

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
29 March 2001 (29.03.2001)

PCT

(10) International Publication Number
WO 01/21829 A1

(51) International Patent Classification⁷: **C12Q 1/00**,
C12N 9/88, 1/20, 15/00, C07H 21/04, C07K 1/00

(72) Inventors: CONTI, Marco; 23 Ryan Court, Stanford, CA
94305 (US). JAISWAL, Bijay, Shankar; 189 Buckthorn
Way, Menlo Park, CA 94025 (US).

(21) International Application Number: PCT/US00/26129

(74) Agent: BORDEN, Paula, A.; Bozicevic, Field & Francis
LLP, Suite 200, 200 Middlefield Road, Menlo Park, CA
94025 (US).

(22) International Filing Date:
21 September 2000 (21.09.2000)

(25) Filing Language: English

(81) Designated States (*national*): AU, CA, JP.

(26) Publication Language: English

(84) Designated States (*regional*): European patent (AT, BE,
CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC,
NL, PT, SE).

(30) Priority Data:
60/155,302 21 September 1999 (21.09.1999) US
60/191,327 22 March 2000 (22.03.2000) US

Published:
— With international search report.

(71) Applicant: THE BOARD OF TRUSTEES OF THE LE-
LAND STANFORD JUNIOR UNIVERSITY [US/US];
900 Welch Road, Suite 350, Palo Alto, CA 94304 (US).

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: POLYNUCLEOTIDES ENCODING HUMAN SOLUBLE ADENYLYL CYCLASE, POLYPEPTIDES ENCODED THEREBY, AND METHODS OF USE THEREOF

(57) Abstract: The invention relates to compositions for and methods of reducing the number of motile sperm in a male. The invention provides methods for identifying substances which inhibit soluble adenylyl cyclase, and which therefore have potential as male contraceptives. The invention further provides isolated polynucleotide sequences encoding human soluble adenylyl cyclase, as well as vectors and host cells comprising the polynucleotide sequences. Further provided are isolated human sAC polypeptides.



WO 01/21829 A1

POLYNUCLEOTIDES ENCODING HUMAN SOLUBLE ADENYLYL CYCLASE,
POLYPEPTIDES ENCODED THEREBY, AND METHODS OF USE THEREOF

GOVERNMENT RIGHTS

5 The United States Government may have certain rights in this application pursuant to
National Institutes of Health Grant No. HD31544.

FIELD OF THE INVENTION

10 This application is in the field of male contraception. More particularly, the invention relates
to inhibition of a soluble adenylyl cyclase.

BACKGROUND OF THE INVENTION

Presently, the major burden of fertility regulation is carried by women, since most available
methods are female methods. Oral contraceptives, implants and injectables, intrauterine devices, tubal
15 ligation, diaphragms, and spermicides are all methods practiced by women. Methods of male
contraception currently available or being developed include vasectomy, condoms, and hormonal
methods.

Spermatogenesis is a differentiation process whereby male germ cells develop into mature
spermatozoa. Leblond, et al.(1952) *Ann. N.Y. Acad. Sci.* 55:548-573; Parvinen (1982) *Endocr. Rev.*
20 3:404-417. Primordial germ cells, derived from primitive ectoderm, are established in the primitive
gonad on embryonic day 10.5 in the mouse. After birth, these cells proliferate extensively, giving rise
to type A spermatogonia which can either replicate as stem cells or differentiate to type B
spermatogonia. At puberty, type B spermatogonia develop into large diploid primary spermatocytes
that undergo two reductive divisions, giving rise to the haploid spermatids. Spermatids evolve into
25 motile spermatozoa through a process referred to as spermiogenesis, characterized by restructuring of
their nuclei and development of flagella.

The cyclic nucleotide-dependent pathway is thought to play an important role in the final
maturation of spermatids. Mice deficient in cAMP responsive element modulator (CREM), a
transacting factor downstream from the cAMP-dependent pathway, are infertile. CREM-null mice
30 display a spermatogenic arrest at the initial spermatid stage, and no spermatozoa are produced. Nantel
et al. (1996) *Nature* 380:159-162; and Blendy et al. (1996) *Nature* 380:162-165. Rather than
differentiating into spermatozoa, spermatids lacking CREM undergo programmed cell death.
Oligoazoospermic men were also found to have a disruption in CREM expression. Lin et al. (1998)
Fertil. Steril. 69:533-538; and Weinbauer et al. (1998) *Mol. Hum. Reprod.* 4:9-15. Among patients
35 with predominant round spermatid maturation arrest, CREM expression is significantly reduced or

undetectable, as revealed by quantitative polymerase chain reaction (PCR) analysis. CREM-negative spermatids fail to progress beyond stage III of spermatogenesis.

Male contraceptive methods that interrupt sperm transport in the male reproductive tract, are not without their complications or long term risks. Comhaire (1994) *Hum. Reprod.* 9:586-590. More complex approaches, such as regimens for the hormonal control of male fertility, have also not been fully satisfactory. Such methods have focused on the suppression of spermatogenesis to the point of azoospermia, a goal which has been difficult to achieve. Baird and Glasier (1993) *N. Engl. J. Med.* 328:1543-1549. This approach, nonetheless, is at the forefront of male contraceptive research, and awaits developments in the pharmacology of oral gonadotropin releasing hormone (GnRH) antagonists before its acceptability can be further advanced. Tom et al. (1992) *J. Clin. Endocrinol. Metab.* 75:476-483.

Other pharmacologic approaches to male contraception have studied the effects of various chemical agents on the functioning of the male reproductive tract. Unfortunately, these studies have not advanced much beyond the search for animal models, since the various side effects of chemicals tested make clinical testing with human males unrealistic. Zaneveld and Waller (1989) *Prog. Clin. Biol. Res.* 302:129-156. The use of the immune response to block contraception has been an important front in efforts to develop more sophisticated contraceptive systems. Unfortunately, such approaches have thus far failed, for a number of reasons. First, male autoimmunity against sperm does not suppress sperm production in men; this is known because such autoimmunity can occur after vasectomy. In addition, it has been found that female immunity against sperm does not necessarily result in infertility. Second, attempts to define the antigenic character of the human sperm surface are still in their infancy, and though epitopes have been identified on human sperm which may have contraceptive potential (Zhang et al. (1992) *Chin. Med. J.* 105:998-1033; Naz (1987) *J. Clin. Invest.* 80:1375-1383), the chemical identity and functions of these antigens themselves are unknown.

It is evident from the foregoing discussion that there is a need for methods of male contraception that are reversible, reliable, and avoid the aforementioned drawbacks. The present invention addresses this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention relates to the use of testis-specific form of adenylyl cyclase, referred to herein as sAC, as a target for male contraception. Inhibition of sAC enzyme activity provides a means for reversibly reducing the number of motile sperm in males, e.g., by inhibiting spermatogenesis, or by reversibly reducing or inhibiting sperm motility, and thus for effecting male contraception. Accordingly, the present invention provides methods for reducing the number of motile sperm in a male. In some embodiments, methods are provided for reducing the number of motile sperm in a

human male. In other embodiments, methods are provided for reducing the number of motile sperm in a non-human male, e.g., in rodent populations. In some embodiments, the present invention provides methods for reducing or inhibiting spermatogenesis in a male. In other embodiments, methods are provided for reducing or inhibiting sperm motility in a male. The invention further provides methods for reversibly achieving male contraception. These methods generally comprising administering to a male a composition comprising an effective amount of a substance which inhibits or reduces sAC enzyme activity.

The invention further provides isolated human sAC polypeptides. Isolated sAC polypeptides are useful for detecting compounds that modulate sAC enzyme activity. Accordingly, the invention further provides methods of identifying substances which modulate sAC enzyme activity. In these methods, sAC polypeptides of any species can be used. In some embodiments, the methods use a human sAC polypeptide and identify compounds that modulate sAC enzyme activity, particularly human sAC enzyme activity. In other embodiments, the methods use rat sAC polypeptides and identify compounds that modulate sAC enzyme activity, particularly rat sAC enzyme activity. The methods generally comprise contacting an sAC enzyme with a substance to be tested, and determining an effect, if any, on sAC enzyme activity. The invention further provides compounds which modulate sAC activity.

The present invention further provides isolated polynucleotides encoding a human soluble adenylyl cyclase (human sAC), and further provides isolated human adenylyl cyclase polypeptides. Polynucleotides and polypeptides of the invention find use in a variety of applications. Isolated human sAC polynucleotides are useful for producing human sAC polypeptides, for identifying modifications in sAC polynucleotides, and for detecting the presence of related polynucleotides. The invention further provides recombinant vectors and isolated host cells comprising polynucleotides of the invention.

These and other objects, advantages, and features of the invention will become apparent to those persons skilled in the art upon reading the details of the polynucleotides, polypeptides, and methods of the invention, as more fully described below.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides the amino acid sequence of human soluble adenylyl cyclase (sAC) (SEQ ID NO:2).

Figures 2A and 2B show an alignment of the amino acid sequences of human and rat sAC (SEQ ID NO 4).

Figures 3A and 3B provide a nucleotide sequence encoding human soluble adenylyl cyclase (SEQ ID NO:1).

Figures 4A-E show an alignment of the nucleotide sequence of exons encoding human sAC with a rat sAC nucleotide sequence (GenBank Accession No. AF081941; SEQ ID NO:3).

Figure 5 is a bar graph depicting activation of sAC adenylyl cyclase activity by bicarbonate.

5 MODES OF CARRYING OUT THE INVENTION

The invention provides methods of detecting substances that modulate soluble adenylyl cyclase (sAC) enzyme activity. In these methods, the sAC can be from any species, including mammalian species. Compositions comprising effective amounts of the substances identified by these methods have the potential to reduce the number of motile sperm, e.g., by inhibiting spermatogenesis or
10 reducing sperm motility, and therefore are of interest as male contraceptives. The invention further provides methods of reducing the number of motile sperm in a male. The invention further provides methods of inhibiting spermatogenesis, as well as methods of reducing sperm motility, in a male. The compositions and methods of the present invention provide means for human male contraception, as well as means for controlling animal populations, e.g., undesired rodent populations.

15 sAC is a cytosolic enzyme found in testis, particularly in spermatids, but not in somatic cells. This enzyme catalyzes the production of adenosine 3',5' cyclic monophosphate (cAMP). cAMP is an important mediator of spermatid differentiation. Accordingly, sAC is a target for substances which inhibit or reduce spermatogenesis, or reduce sperm motility, and which therefore have the potential to function as male contraceptives.

20 sAC, as described herein, is characterized by one or more of the following features: (1) it is expressed in high levels in the testis, particularly in spermatids, and, is either absent from, or is present at only low levels in somatic cells; (2) unlike membrane-associated adenylyl cyclases, it is not associated with G proteins, and thus is insensitive to G protein regulation (e.g., it is not activatable by forskolin); (3) it is found in the cytosol (i.e., is not an integral membrane protein); (4) its reaction
25 product is cAMP; (5) it exhibits an approximately 10-fold lower affinity for its substrate, adenosine triphosphate (ATP) than membrane-associated adenylyl cyclases ($K_m \approx 1$ mM for sAC, compared with $K_m \approx 100$ μ M for membrane-associated adenylyl cyclases); (6) its adenylyl cyclase activity is dependent on the presence of divalent manganese or magnesium ions; and (7) its adenylyl cyclase activity is activated by bicarbonate ions, e.g., its adenylyl cyclase activity is activated by sodium
30 bicarbonate.

The present invention further provides isolated polynucleotides comprising sequences which encode human soluble adenylyl cyclase (human sAC). Isolation of a human polynucleotide encoding sAC, and determination of its sequence, allows production of human sAC polypeptides.

Before the present invention is described, it is to be understood that this invention is not
35 limited to particular embodiments described, as such may, of course, vary. It is also to be understood

that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth
5 of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits
10 are included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although
15 any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

It must be noted that as used herein and in the appended claims, the singular forms "a", "and",
20 and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an agent" includes a plurality of such agents and reference to "the polynucleotide" includes reference to one or more polynucleotides and equivalents thereof known to those skilled in the art, and so forth.

The publications discussed herein are provided solely for their disclosure prior to the filing
25 date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

30 General Methods

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. *See e.g., Sambrook, Fritsch, and Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition (1989), Oligonucleotide
35 Synthesis (M. J. Gait Ed., 1984), Animal Cell Culture (R. I. Freshney, Ed., 1987), the series Methods*

in Enzymology Academic Press, Inc.); Gene Transfer Vectors for Mammalian Cells (J. M. Miller and M. P. Calos Eds. 1987), Handbook of Experimental Immunology, (D. M. Weir and C. C. Blackwell, Eds.); Current Protocols in Molecular Biology, (F. M. Ausubel, et al., Eds. 1987, and updates); and Current Protocols in Immunology (J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach and W. Strober Eds. 1991).

Definitions

The terms "polynucleotide" and "nucleic acid", used interchangeably herein, refer to a polymeric forms of nucleotides of any length, either ribonucleotides or deoxynucleotides. Thus, this term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. The backbone of the polynucleotide can comprise sugars and phosphate groups (as may typically be found in RNA or DNA), or modified or substituted sugar or phosphate groups. Alternatively, the backbone of the polynucleotide can comprise a polymer of synthetic subunits such as phosphoramidites and thus can be an oligodeoxynucleoside phosphoramidate or a mixed phosphoramidate-phosphodiester oligomer. Peyrottes et al. (1996) Nucl. Acids Res. 24:1841-1848; Chaturvedi et al. (1996) Nucl. Acids Res. 24:2318-2323. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs, uracyl, other sugars, and linking groups such as fluororibose and thioate, and nucleotide branches. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. Other types of modifications included in this definition are caps, substitution of one or more of the naturally occurring nucleotides with an analog, and introduction of means for attaching the polynucleotide to proteins, metal ions, labeling components, other polynucleotides, or a solid support.

The terms "polypeptide" and "protein", used interchangeably herein, refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones. The term includes fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence, fusions with heterologous and homologous leader sequences, with or without N-terminal methionine residues; immunologically tagged proteins; and the like.

A polynucleotide or polypeptide has a certain percent "sequence identity" to another polynucleotide or polypeptide, meaning that, when aligned, that percentage of bases or amino acids are the same when comparing the two sequences. Sequence similarity can be determined in a number of different manners. To determine sequence identity, sequences can be aligned using the methods and computer programs, including BLAST, available over the world wide web at

<http://www.ncbi.nlm.nih.gov/BLAST/>. Another alignment algorithm is FASTA, available in the Genetics Computing Group (GCG) package, from Madison, Wisconsin, USA, a wholly owned subsidiary of Oxford Molecular Group, Inc. Other techniques for alignment are described in Methods in Enzymology, vol. 266: Computer Methods for Macromolecular Sequence Analysis (1996), ed.

5 Doolittle, Academic Press, Inc., a division of Harcourt Brace & Co., San Diego, California, USA. Of particular interest are alignment programs that permit gaps in the sequence. The Smith-Waterman is one type of algorithm that permits gaps in sequence alignments. See Meth. Mol. Biol. 70: 173-187 (1997). Also, the GAP program using the Needleman and Wunsch alignment method can be utilized to align sequences. See J. Mol. Biol. 48: 443-453 (1970)

10 Of interest is the BestFit program using the local homology algorithm of Smith Waterman (Advances in Applied Mathematics 2: 482-489 (1981) to determine sequence identity. The gap generation penalty will generally range from 1 to 5, usually 2 to 4 and in many embodiments will be 3. The gap extension penalty will generally range from about 0.01 to 0.20 and in many instances will be 0.10. The program has default parameters determined by the sequences inputted to be compared.

15 Preferably, the sequence identity is determined using the parameters determined by the program. This program is available also from Genetics Computing Group (GCG) package, from Madison, Wisconsin, USA.

Another program of interest is the FastDB algorithm. FastDB is described in Current Methods in Sequence Comparison and Analysis, Macromolecule Sequencing and Synthesis, Selected

20 Methods and Applications, pp. 127-149, 1988, Alan R. Liss, Inc. Percent sequence identity is calculated by FastDB based upon the following parameters:

Mismatch Penalty: 1.00;
 Gap Penalty: 1.00;
 Gap Size Penalty: 0.33; and
 25 Joining Penalty: 30.0.

One parameter for determining percent sequence identity is the "percentage of the alignment region length" where the strongest alignment is found.

The percentage of the alignment region length is calculated by counting the number of residues of the individual sequence found in the region of strongest alignment. This number is divided by the

30 total residue length of the target or query polynucleotide sequence to find a percentage. An example is shown below:

Target sequence:	GCGCGAAATACTCACTCGAGG
Query sequence:	TATAGCCCTAC.CACTAGAGTCC
	1 5 10 15

The region of alignment begins at residue 9 and ends at residue 19. The total length of the target sequence is 20 residues. The percent of the alignment region length is 11 divided by 20 or 55%, for example.

Percent sequence identity is calculated by counting the number of residue matches between the target and query polynucleotide sequence and dividing total number of matches by the number of residues of the target or query sequence found in the region of strongest alignment. For the example above, the percent identity would be 10 matches divided by 11 residues, or approximately, 90.9%.

The percent of the alignment region length is typically at least about 55% of total length of the sequence, more typically at least about 58%, and even more typically at least about 60% of the total residue length of the sequence. Usually, percent length of the alignment region can be as great as about 62%, more usually as great as about 64% and even more usually as great as about 66%.

"Recombinant," as used herein, means that a particular DNA sequence is the product of various combinations of cloning, restriction, and ligation steps resulting in a construct having a structural coding sequence distinguishable from homologous sequences found in natural systems. Generally, DNA sequences encoding the structural coding sequence, for example cytokines, can be assembled from cDNA fragments and short oligonucleotide linkers, or from a series of oligonucleotides, to provide a synthetic gene which is capable of being expressed in a recombinant transcriptional unit. Such sequences are preferably provided in the form of an open reading frame uninterrupted by internal nontranslated sequences, or introns, which are typically present in eukaryotic genes. Genomic DNA containing the relevant sequences could also be used. Sequences of non-translated DNA may be present 5' or 3' from the open reading frame, where such sequences do not interfere with manipulation or expression of the coding regions. Thus, the term "recombinant" polynucleotide or nucleic acid refers to one which is not naturally occurring, or is made by the artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by either chemical synthesis means, or by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques. Such is usually done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a sequence recognition site. Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a desired combination of functions.

By "antisense polynucleotide" is mean a polynucleotide having a nucleotide sequence complementary to a given polynucleotide sequence (e.g., a polynucleotide sequence encoding a soluble adenylyl cyclase polypeptide) including polynucleotide sequences associated with the transcription or translation of the given polynucleotide sequence (e.g., a promoter of a polynucleotide encoding sAC polypeptide), where the antisense polynucleotide is capable of hybridizing to a human sAC polypeptide-encoding polynucleotide sequence. Of particular interest are antisense polynucleotides

capable of inhibiting transcription and/or translation of a sAC-encoding polynucleotide either in vitro or in vivo.

"Peptide nucleic acid" as used herein refers to a molecule which comprises an oligomer to which an amino acid residue, such as lysine, and an amino group have been added. These small molecules, also designated anti-gene agents, stop transcript elongation by binding to their complementary (template) strand of nucleic acid (Nielsen et al. (1993) *Anticancer Drug Des.* 8:53-63).

The term "specific binding" with respect to antibody-antigen interactions, is a term well understood in the art and indicates that a given antibody binds to an antigenic determinant (epitope) which was used as an immunogen, and does not substantially bind to an unrelated antigenic determinant.

As used herein the term "isolated" is meant to describe a polynucleotide, a polypeptide, an antibody, or a host cell that is in an environment different from that in which the polynucleotide, the polypeptide, the antibody, or the host cell naturally occurs. As used herein, the term "substantially purified" refers to a compound (e.g., either a polynucleotide or a polypeptide or an antibody) that is removed from its natural environment and is at least 60% free, preferably 75% free, and most preferably 90% free from other components with which it is naturally associated.

The term "host cell" includes an individual cell or cell culture which can be or has been a recipient of any recombinant vector(s) or isolated polynucleotide of the invention. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation and/or change. A host cell includes cells transfected or infected *in vivo* or *in vitro* with a recombinant vector or a polynucleotide of the invention. A host cell which comprises a recombinant vector of the invention is a "recombinant host cell".

The term "immunologically active" defines the capability of the natural, recombinant or synthetic human sAC polypeptide, or any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies. As used herein, "antigenic amino acid sequence" means an amino acid sequence that, either alone or in association with a carrier molecule, can elicit an antibody response in a mammal.

By "transformation" is meant a permanent or transient genetic change induced in a cell following incorporation of new DNA (i.e., DNA exogenous to the cell). Genetic change can be accomplished either by incorporation of the new DNA into the genome of the host cell, or by transient or stable maintenance of the new DNA as an episomal element. Where the cell is a mammalian cell, a permanent genetic change is generally achieved by introduction of the DNA into the genome of the cell.

By "construct" is meant a recombinant nucleic acid, generally recombinant DNA, that has been generated for the purpose of the expression of a specific nucleotide sequence(s), or is to be used in the construction of other recombinant nucleotide sequences.

A "transcriptional control region" (sometimes referred to as a "transcriptional regulatory region") encompasses all the elements necessary for transcription, and may include elements necessary for transcription. Thus, a transcriptional control region includes at least the promoter sequence, and may also include other regulatory sequences such as enhancers, and transcription factor binding sites.

A "transcriptional control region heterologous to a coding region" is one that is not normally associated with the coding region in nature.

"Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For instance, a promoter is operably linked to a coding sequence if the promoter effects its transcription or expression.

"Regulatory sequences" refer to those sequences normally associated with (for example within 50 kb) of the coding region of a locus which affect the expression of the gene (including transcription of the gene, and translation, splicing, stability, or the like of the messenger RNA). Regulatory sequences include, inter alia, promoters, enhancers, splice sites and polyadenylation sites.

The term "biological sample" encompasses a variety of sample types obtained from an organism and can be used in a diagnostic or monitoring assay. The term encompasses blood and other liquid samples of biological origin, solid tissue samples, such as a biopsy specimen or tissue cultures or cells derived therefrom and the progeny thereof. The term encompasses samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components. The term encompasses a clinical sample, and also includes cells in cell culture, cell supernatants, cell lysates, serum, plasma, biological fluids, and tissue samples.

By "individual" or "subject" or "patient" is meant any mammalian subject for whom diagnosis, treatment, or therapy is desired, particularly humans, particularly male humans. Other subjects may include cattle, dogs, cats, guinea pigs, rabbits, rats, mice, horses, and so on.

METHODS OF THE INVENTION

The present invention provides methods for reducing the number of motile sperm in a male. The invention further provides methods for inhibiting or reducing spermatogenesis in a male subject, and for reducing sperm motility in a male subject. Such methods are useful in male contraception. The male may be a human male, or may be a non-human male, including, for example, a male in a non-human animal population, such as an undesired rodent population.

The present invention also provides methods for identifying agents which modulate a level of sAC enzyme activity; methods for identifying agents which modulate a level of sAC mRNA; methods for identifying agents which modulate a level of sAC polypeptide.

5 The present invention further provides methods for detecting the presence and/or measuring an amount of a human sAC mRNA in a biological sample; and methods of detecting the presence of and/or measuring an amount of human sAC polypeptide in a biological sample.

Methods of reducing the number of motile sperm

The present invention provides methods of reducing the number of motile sperm in a male.

10 Reduction of the number of motile sperm can occur by inhibiting spermatogenesis, and/or by inhibiting sperm motility, in a male. The methods generally comprise administering to the individual a composition comprising an effective amount of a substance that inhibits sAC adenylyl cyclase activity, or that inhibits production of sAC mRNA and/or sAC polypeptide, i.e., administration of the substance results in reduced levels of active sAC. These methods are useful in achieving male contraception. A

15 reduction in numbers of motile sperm may be an indication that the substance reduces spermatogenesis, or that the substance reduces sperm motility. The invention further provides methods for reducing sperm motility in a male. The invention further provides methods for reducing or inhibiting spermatogenesis in a male.

Substances of interest which inhibit sAC adenylyl cyclase are those that inhibit (reduce)

20 spermatogenesis, e.g., that reduce the number of sperm produced by a male, particularly the number of motile sperm produced by a male; and substances that inhibit sperm motility. Inhibition of spermatogenesis can be determined by determining the number of motile and non-motile sperm in a sperm sample. Generally, an "effective amount" of a substance is an amount that is effective in reducing the number of motile sperm produced by the male to which the substance is administered. An

25 effective amount of a substance results in a reduction of motile sperm production, as measured in a sperm sample from the subject, to less than about 10 million spermatozoa/mL, typically less than about 5 million spermatozoa/mL, usually less than about 1 million spermatozoa/mL. World Health Organization standards can also be used to assess the effect of a substance on fertility. The WHO standard for oligospermia is 20×10^6 sperm/ml, and for severe oligospermia is $3-5 \times 10^6$ sperm/ml,

30 with fewer than 50% motility in each case. Motility can be determined by any known means, including, but not limited to, CASA, or computer-assisted sperm analysis, which measures various sperm motility parameters over time.

The invention further provides methods of male contraception. In some embodiments, the methods are reversible, e.g., when the substance is no longer administered to the individual, the level of

fertility (i.e., motile sperm production) which existed in the individual before administration of the substance is restored to the individual, usually within a few days, or weeks, up to about a few months. The methods generally comprise administering to a male a pharmaceutical composition comprising an effective amount of a substance which preferentially inhibits soluble adenylyl cyclase, or which
5 preferentially reduces sAC mRNA synthesis and/or sAC polypeptide synthesis. A substance which preferentially inhibits soluble adenylyl cyclase, or which preferentially reduces sAC mRNA synthesis and/or sAC polypeptide synthesis, is one that inhibits the testis-specific soluble adenylyl cyclase, or which reduces testis-specific sAC mRNA synthesis and/or testis specific sAC polypeptide synthesis, as described herein, but which does not substantially reduce activity of other, non-sAC, enzymes, or
10 which does not substantially reduce synthesis of other, non-sAC, mRNA species or synthesis of other, non-sAC, proteins. Thus, for example, transmembrane, G protein-coupled, adenylyl cyclase activity and/or synthesis is substantially not affected by the substance, e.g., the activity and/or synthesis of a transmembrane, G protein-coupled adenylyl cyclase retains at least about 90% of its activity and/or synthesis in the presence of the substance.

15 The methods can be applied to a wide variety of species, including mammals, reptiles, amphibians, insects, arachnids, etc. Of particular interest are methods of reducing sperm production (e.g., reducing the number of motile sperm) in human males. Also of interest are methods of reducing sperm production in unwanted rodent populations. Also of interest are methods of reducing sperm production in feral mammalian populations (e.g., feral cats, feral dogs), where it is desired to reduce
20 the number of offspring of such animals. Of further interest are methods of reducing spermatogenesis in unwanted insect populations, including, but not limited to, mosquitoes, fleas, weevils, locusts, and the like.

Reduction of sperm production can be species-specific, e.g., the substance administered can be one that inhibits sperm production only in human males. Alternatively, the substance can be one
25 that reduces sperm production in more than one species, e.g., of interest are substances that inhibit sperm production in both rats and mice.

Whether spermatogenesis has been inhibited or reduced can be determined by any known method. For example, a sperm sample can be obtained from the individual at a suitable time after the composition has been administered to the individual. The sperm sample can be a freshly collected
30 ejaculate; seminal plasma-free sperm, e.g., sperm that has been substantially isolated from other semen components, such as by centrifugation. The total number of motile and non-motile sperm is counted. Sperm can be quantitated based on detecting a component of sperm, or can be performed visually, e.g., counting the number of sperm visually under a microscope.

For determining whether a particular male contraceptive has been effective, the test must be
35 particularly sensitive. For example, the VasScore™ test, can successfully distinguish colors based on a

quantity of sperm protein that corresponds to a sperm concentration in the sample that is greater than or equal to about 100,000 spermatozoa/mL. If the sperm sample is semen, it is preferred that the semen is liquefied prior to analysis. Although a semen sample will typically liquefy on its own at room temperature in about 30-60 minutes, preferably the semen sample is liquefied in less than 30 minutes and most preferably it is liquefied in 5-15 minutes, based on contact with a liquefaction reagent. Examples of appropriate liquefaction reagents include non-enzymatic reagents, such as dithiothreitol (DTT) (e.g. 1-5 mg/ml) and enzymes such as chymotrypsin and pronase (e.g. 5-15 mg/ml.)

Methods for determining sperm fertility potential include those involving quantitating a particular sperm target or component as an indication of the number of sperm present in a sperm containing sample. Examples of appropriate targets or components include sperm proteins (e.g. sperm flagellar proteins, glycolytic enzymes, antioxidant enzymes (e.g. glutathione peroxidase or superoxide dismutase), nuclear proteins, acrosomal proteins, α -tubulin, lactate dehydrogenase (LDH-X), protamine (sperm histones), acrosomal proteins (e.g. acrosin) or mitochondrial proteins); sperm lipids (e.g. cholesterol, phospholipids, glycolipids, triglycerides, phosphatidylglycerols, seminolipids, and fatty acids, particularly docosahexaenoic acid, which is one of the few fatty acids found in sperm); nucleic acids or a mixture of sperm components (e.g. thiazine blue reacts with sperm proteins, lipids and other sperm components).

An example of a test for male fertility which can be used in the present invention is described in U.S. Patent No. 5,935,800.

Suitable sperm reagents include labeled (e.g. dye or tracer labeled) or unlabeled reagents that specifically stain based on interaction with a sperm component (e.g. sperm protein, sperm lipid, sperm nucleic acid, sperm carbohydrate and/or other sperm component (e.g. mitochondria, nucleus). For example, Protein Reagent (0.3% tetrabromophenol, Miles Scientific, Connecticut) colors sperm based on interaction with sperm protein. Rhodamine 123 colors sperm by accumulating within sperm mitochondria. Detectably labeled protein A specifically colors sperm by binding to sperm bound antibodies. Propidium iodide and eosin specifically color sperm by interacting with sperm nucleic acids. However, both reagents can only diffuse into cells which have been permeabilized (e.g. using a detergent). Acridine orange, on the other hand, can specifically color unpermeabilized sperm based on interaction with decondensed sperm chromatin (but not condensed sperm chromatin). Sperm chromatin can be decondensed prior to staining with acridine orange by reducing sperm protamines (i.e. sperm histones). A preferred reagent for reducing sperm protamines is dithiothreitol (DTT). Other reagents, which specifically interact with a semen or washed sperm sample, include gold particles (which have an intrinsic pink color and react with proteins) thiazine blue, tetrabromophenol and rhodamine (red-colored and reacts with lipids). Other preferred sperm reagents include sperm antibodies, such as labeled (e.g. enzyme, tracer (e.g. radioactive), dye or color particle labeled) or unlabeled anti-sperm

antibodies (e.g. anti-human sperm polyclonal antibody; Arnel Products Co., Inc, Cherokee Station, New York, N.Y.; Chemicon International Inc., Temecula, Calif.) or labeled or unlabeled antibodies against a sperm component (e.g. a sperm protein or sperm lipid). Preferred antibodies include anti-human sperm polyclonal antibodies and antibodies specific to an epitope of the sperm flagellum, nuclear proteins, glycolytic enzymes, acrosome etc.).

One method for quantitating sperm involves (i) incubating a sperm sample with colored particles containing anti-sperm antibodies for an appropriate period of time to allow the sperm antigens to react with the antibody bound colored particles; ii) filtering the sample of step i), so that sperm/colored particle/antibody complex is retained on the filter and unbound, colored particle/antibody and seminal plasma protein passes through the filter; and iii) visualizing the color intensity on the filter as an indication of the number of sperm on the filter. For example, if colored particles are used, sperm/colored particle/antibody complexes can be quantitated by comparing the color of the filter to a color chart, which depicts various color possibilities for various quantities of sperm.

In order to use antibodies or reagents that may also react with seminal plasma components present in a sperm containing sample (e.g. ejaculate), sperm can first be isolated. Seminal plasma-free sperm can then be contacted with anti-sperm antibody coated colored particles. After a sufficient period of time to allow antibodies and antigens to react, unbound antibody coated colored particles can be removed from the mixture and sperm/colored particle/antibody complex detected and quantitated. Alternatively, sperm can be quantitated by detecting the appearance of agglutination in a drop of sample following addition of anti-sperm antibodies with bound latex particles.

Immunodetection of an antigenic indicator of sperm in a sample can be accomplished using any of a number of competitive or non-competitive assay procedures. In general, competitive immunoassays are performed by adding the antigen to be detected to a sperm containing sample, so that the sperm and the antigen compete for a limited number of antibody binding sites resulting in the formation of sperm-antibody and labeled antigen-antibody complexes. By maintaining the concentration of labeled antigen and antibody constant, the amount of labeled antibody complex formed is inversely proportional to the amount of sperm present in the sample. A quantitative determination of the sperm can therefore be made based on the labeled antibody complex.

Competitive assays can be homogeneous (i.e. not requiring separation of antibody bound tracer (e.g. labeled antigen) from free tracer, since the antigen-antibody interaction causes, directly or indirectly, a measurable change in the signal obtained from the label group of the tracer). Alternatively, competitive assays can be heterogeneous (i.e. requiring separation of bound tracer from free tracer prior to determining the amount of ligand in the sample).

In contrast to competitive immunoassays, non-competitive assays involve incubating a sperm containing sample with an immobilized sperm antibody for a period of time sufficient to reach equilibrium with regard to the formation of antibody-sperm conjugates. The sperm antibody can be directly or indirectly labeled. For example, indirect labeling can be carried out after a

5 wash step to remove unbound sperm by contacting the immobilized antibody-sperm complexes with a second, labeled antibody that is specific for the antibody-sperm complex. Following a second wash step to remove unbound second antibody, the amount of bound second antibody can be detected and measured as an indication of bound sperm.

10 Methods of identifying substances that modulate sAC enzyme activity

The present invention provides methods of identifying agents which modulate an adenylyl cyclase activity of an sAC polypeptide. In some embodiments, the methods are cell-based methods. In other embodiments, the methods are cell-free methods. The term "modulate" encompasses an increase or a decrease in the measured adenylyl cyclase activity when compared to a suitable control.

15 The methods generally comprise:

a) contacting a substance to be tested with a sample containing an sAC polypeptide; and

b) assaying an adenylyl cyclase activity of the human sAC polypeptide in the presence of the substance, wherein an increase or a decrease in adenylyl cyclase activity in comparison to sAC adenylyl cyclase activity in a suitable control is an indication that the substance modulates an adenylyl
20 cyclase activity of the sAC.

The sAC polypeptide used in these methods is one that is preferentially produced in testis, in particular, in spermatids, of the species from which it was derived. sAC polypeptides are produced preferentially in those cells which preferentially express sAC mRNA. The term "preferentially expressed in spermatids" indicates that sAC mRNA is expressed at a level generally at least about 2-
25 fold, usually at least about 10-fold, typically at least about 20-fold or more higher in spermatids than in somatic cells of the body. A convenient reference cell is the Sertoli cell, which does not express sAC mRNA. Whether an sAC mRNA is expressed preferentially in spermatids can be readily determined by those skilled in the art, by determining the level of sAC mRNA in spermatids, in comparison with sAC mRNA levels in a somatic cell, including, but not limited to, Sertoli cells.

30 Methods of measuring mRNA levels are known in the art, and any of these methods are suitable for use herein. Suitable methods include Northern analysis, and PCR.

The sAC polypeptide can be a full-length polypeptide (e.g., has an amino acid sequence of the same length as that found in its natural environment, or "wild-type" sequence), but need not be full-length, as long as the sAC polypeptide retains measurable adenylyl cyclase activity. Furthermore, the
35 sAC polypeptide used in these assays may also contain alterations in amino acid sequence compared to

the wild-type sequence, wherein such alterations may confer a desirable property, including, but not limited to, enhanced stability *in vitro*, and the like. The sAC polypeptide may further be a fusion protein comprising an sAC polypeptide and a heterologous polypeptide, e.g. a non-sAC polypeptide, including, but not limited to, an epitope to facilitate recovery of the sAC from the sample, and the like.

5 In some embodiments, the sAC polypeptide is a human sAC polypeptide of the invention. In other embodiments, the sAC polypeptide is one derived from non-human species, including, but not limited to, a non-human mammalian species, such as rat. For example, polypeptides comprising the sequences set forth in SEQ ID NO:2, which depicts human sAC, and SEQ ID NO:4, which depicts a rat sAC amino acid sequence, or fragments of these sequences which retain adenylyl cyclase activity,
10 can be used.

Where the method is a cell-free assay method, sAC enzyme may be, but need not be, substantially purified. In general, the enzyme should be isolated from the source of the enzyme in those instances where one or more components found in the source of the enzyme interfere with enzyme activity or measurement of enzyme activity. The sample can be a cell lysate comprising sAC,
15 or the sample can comprise sAC which is purified to any degree. As non-limiting examples, the sample can be: a cell lysate of a mammalian cell line which has been transfected with a recombinant vector ("construct") which encodes and expresses sAC polypeptide having adenylyl cyclase activity; and sAC which has been purified from a biological source.

sAC adenylyl cyclase activity can be measured in a cell-free system (e.g., in cell lysates, or in
20 a sample comprising isolated sAC), using any known assay, such as those described in Buck et al. (1999) *Proc. Natl. Acad. Sci. USA* 96:79-84; Steiner et al. (1974) *Methods Enzymol.* 38:96-105; and Harper et al. (1975) *J. Cyclic Nucleotide Res.* 1:207-218. sAC activity is generally measured by measuring an amount of cAMP produced. sAC activity may be measured in the presence of labeled substrate ATP, thereby generating labeled product cAMP. For example, [α - 32 P]ATP, or any other
25 labeled form of ATP can be used, as long as the product cAMP is detectably labeled. Typically, these assays are conducted in the presence of 5 mM MnCl₂ and 5 mM [α - 32 P]ATP. sAC activity may also be measured by measuring a level of cAMP using cAMP-specific antibodies, using, e.g., an ELISA or an RIA. Commercially available kits may also be used.

The following is a non-limiting example of how sAC activity can be measured in cell lysates.
30 Mammalian cells are transfected with a recombinant vector comprising a nucleotide sequence which encodes an sAC. After two days, cells are labeled overnight with 5 mM [α - 32 P]ATP (specific activity 4×10^4 cpm/nmol). The next day, cells are suspended in 200 μ l of lysis buffer (50 mM Tris (pH 7.5), 1 mM EDTA, 1 mM dithiothreitol, 0.1 mg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride) and disrupted by sonication for 1 minute on ice. Extracts are centrifuged at 100,000 x g for 10 minutes.
35 The supernatant, i.e., cytosolic fraction, is assayed for sAC activity in the presence of 5 mM MnCl₂.

Where the method is a cell-based assay method, a cell which synthesizes sAC is contacted with the compound being tested, and, after a suitable time, the cells are lysed, and sAC activity is measured in the cell lysates. Alternatively, the cells need not be lysed in order to measure sAC adenylyl cyclase activity. In these embodiments, cAMP levels are measured in intact cells. As a non-limiting example, a construct comprising nucleotide sequence encoding an sAC polypeptide is introduced into a cell line, e.g., COS cells, HEK293 cells, or MA-10 cells, such that sAC is expressed in the cells. For these assays, the sAC coding region may be under control of an endogenous promoter, or, alternatively, under control of an inducible promoter. Inducible promoters are known in the art, and can be used in such a construct. Suitable inducible promoters include, but are not limited to, a hormone-inducible promoter. When an inducible promoter is used, the inducer is added to the cell culture before, or simultaneously with, the substance being tested. Controls include a culture to which no inducer has been added, as well as a culture to which inducer, but no substance being tested, is added.

Alternatively, endogenous sAC activity can be measured in intact cells.

sAC adenylyl cyclase activity can be measured in an intact cell using any known assay, such as those described above, wherein accumulation of labeled cAMP is measured in intact cells. A non-limiting example of such an assay is a fluorescence energy transfer assay, as described in U.S. Patent No. 5,439,797. Commercially available kits may also be used.

Assays such as those described herein are amenable to high through-put screening assays.

For example, isolated sAC, or cells comprising endogenous sAC, or cells expressing a construct encoding sAC, each in separate well of a microtiter plate, e.g., can be contacted with a large number of test compounds at a time, thereby allowing automation.

The term "agent" is used interchangeably herein with the terms "substance" and "compound". An "agent which modulates an adenylyl cyclase activity of a sAC polypeptide", as used herein, describes any molecule, e.g. protein; peptide; natural or synthetic inorganic or organic compound, or pharmaceutical, with the capability of altering an adenylyl cyclase activity of a sAC polypeptide, as described herein. Generally a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e. at zero concentration or below the level of detection.

Candidate agents encompass numerous chemical classes, and may be natural or synthetic inorganic or organic molecules, which may be small organic compounds having a molecular weight of more than 50 and less than about 2,500 daltons. Candidate agents may comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures

and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides and oligopeptides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, glycosylation, amidification, etc. to produce structural analogs.

A variety of other reagents may be included in the screening assay. These include reagents like salts, neutral proteins, e.g. albumin, detergents, etc that are used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Reagents that improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc. may be used. The mixture of components are added in any order that provides for the requisite binding. Incubations are performed at any suitable temperature, typically between 4 and 40°C. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high-throughput screening. Typically between 0.1 and 1 hours will be sufficient.

Adenylyl cyclase activity can be measured using any adenylyl cyclase assay known in the art. An agent which modulates an adenylyl cyclase activity of a human sAC polypeptide increases or decreases the activity at least about 10%, more preferably at least about 25%, more preferably at least about 50%, more preferably at least about 100%, or 2-fold, more preferably at least about 5-fold, more preferably at least about 10-fold or more when compared to a suitable control.

Agents which increase or decrease an adenylyl cyclase activity of a human sAC polypeptide to the desired extent may be selected for further study, and assessed for bioavailability, cellular availability, cytotoxicity, biocompatibility, etc.

Agents which decrease an adenylyl cyclase activity of a human sAC polypeptide may find use in methods for inhibiting spermatogenesis, and thus may find use as male contraceptives.

Methods of detecting agents which modulate a level of sAC mRNA and/or sAC polypeptide

A wide variety of cell-based assays may be used for identifying agents which modulate levels of human sAC mRNA, using, for example, a mammalian cell transformed with a construct comprising

a human sAC-encoding cDNA such that the cDNA is overexpressed. In these assays, a level of sAC mRNA or polypeptide is measured.

Alternatively, a construct comprising a cAMP-sensitive promoter operably linked to a reporter gene, e.g., luciferase, can be used. In such, a level of sAC produced is measured in the presence and absence of a substance being tested. Inhibition of sAC mRNA and/or polypeptide production is measured in terms of a decrease in the level of signal produced by the reporter gene product. An example of such a construct is one which comprises a cAMP-responsive element (CRE) operably linked to reporter gene encoding luciferase. A multiplicity of CRE can be used, e.g., three CRE in tandem.

Accordingly, the present invention provides a method for identifying an agent, particularly a biologically active agent, that modulates a level of human sAC expression in a cell, the method comprising: combining a candidate agent to be tested with a cell comprising a nucleic acid which encodes a human sAC polypeptide; and determining the effect of said agent on human sAC expression. "Modulation" of human sAC expression levels includes increasing the level and decreasing the level of human sAC mRNA and/or human sAC polypeptide encoded by the human sAC polynucleotide when compared to a control lacking the agent being tested. An increase or decrease of about 1.25-fold, usually at least about 1.5-fold, usually at least about 2-fold, usually at least about 5-fold, usually at least about 10-fold or more, in the level (i.e., an amount) of human sAC mRNA and/or polypeptide following contacting the cell with a candidate agent being tested, compared to a control to which no agent is added, is an indication that the agent modulates human sAC expression.

An agent being tested for its effect on human sAC expression is assessed for any cytotoxic activity it may exhibit toward the cell used in the assay, using well-known assays, including, but not limited to, trypan blue dye exclusion, an MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2 H-tetrazolium bromide]) assay, and the like. Agents that do not exhibit cytotoxic activity are considered candidate agents.

The cells used in the assay are usually mammalian cells, including, but not limited to, rodent cells and human cells. The cells may be primary cultures of spermatids, or may be immortalized cell lines, e.g., COS cells, MA-10 cells, HEK293 cells, and the like.

Human sAC mRNA and/or polypeptide whose levels are being measured can be encoded by an endogenous human sAC polynucleotide, or the human sAC polynucleotide can be one that is comprised within a recombinant vector and introduced into the cell, i.e., the human sAC mRNA and/or polypeptide can be encoded by an exogenous human sAC polynucleotide. For example, a recombinant vector may comprise an isolated human human sAC transcriptional regulatory sequence, such as a promoter sequence, operably linked to a reporter gene (e.g., β -galactosidase, CAT, luciferase, or other gene that can be easily assayed for expression). In these embodiments, the method for identifying an

agent that modulates a level of human sAC expression in a cell, comprises: combining a candidate agent to be tested with a cell comprising a nucleic acid which comprises a human sAC gene transcriptional regulatory element operably linked to a reporter gene; and determining the effect of said agent on reporter gene expression. A recombinant vector may comprise an isolated human sAC transcriptional regulatory sequence, such as a promoter sequence, operably linked to sequences coding for a human sAC polypeptide; or the transcriptional control sequences can be operably linked to coding sequences for a human sAC fusion protein comprising human sAC polypeptide fused to a polypeptide which facilitates detection. In these embodiments, the method comprises combining a candidate agent to be tested with a cell comprising a nucleic acid which comprises a human sAC gene transcriptional regulatory element operably linked to a human sAC polypeptide-coding sequence; and determining the effect of said agent on human sAC expression, which determination can be carried out by measuring an amount of human sAC mRNA, human sAC polypeptide, or human sAC fusion polypeptide produced by the cell.

Cell-based assays generally comprise the steps of contacting the cell with an agent to be tested, forming a test sample, and, after a suitable time, assessing the effect of the agent on human sAC expression. A control sample comprises the same cell without the candidate agent added. human sAC expression levels are measured in both the test sample and the control sample. A comparison is made between human sAC expression level in the test sample and the control sample. human sAC expression can be assessed using conventional assays. For example, when a mammalian cell line is transformed with a construct that results in expression of human sAC, human sAC mRNA levels can be detected and measured, as described above, or human sAC polypeptide levels can be detected and measured, as described above. A suitable period of time for contacting the agent with the cell can be determined empirically, and is generally a time sufficient to allow entry of the agent into the cell and to allow the agent to have a measurable effect on human sAC mRNA and/or polypeptide levels.

Generally, a suitable time is between 10 minutes and 24 hours, more typically about 1-8 hours. Methods of measuring human sAC mRNA levels are known in the art, several of which have been described above, and any of these methods can be used in the methods of the present invention to identify an agent which modulates human sAC mRNA level in a cell, including, but not limited to, a PCR, such as a PCR employing detectably labeled oligonucleotide primers, and any of a variety of hybridization assays. The use of the polymerase chain reaction is described in Saiki et al. (1985) *Science* 239:487, and a review of current techniques may be found in Sambrook, *et al.* Molecular Cloning: A Laboratory Manual, CSH Press 1989, pp.14.2-14.33. A detectable label may be included in the amplification reaction. Suitable labels include fluorochromes, *e.g.* fluorescein isothiocyanate (FITC), rhodamine, Texas Red, phycoerythrin, allophycocyanin, 6-carboxyfluorescein (6-FAM), 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein (JOE), 6-carboxy-X-rhodamine (ROX),

6-carboxy-2',4',7',4,7-hexachlorofluorescein (HEX), 5-carboxyfluorescein (5-FAM) or N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), radioactive labels, *e.g.* ^{32}P , ^{35}S , ^3H ; *etc.* The label may be a two stage system, where the amplified DNA is conjugated to biotin, haptens, *etc.* having a high affinity binding partner, *e.g.* avidin, specific antibodies, *etc.*, where the binding partner is conjugated to a detectable label. The label may be conjugated to one or both of the primers. Alternatively, the pool of nucleotides used in the amplification is labeled, so as to incorporate the label into the amplification product.

Similarly, human sAC polypeptide levels can be measured using any standard method, several of which have been described herein, including, but not limited to, an immunoassay such as ELISA, for example an ELISA employing a detectably labeled antibody specific for a human sAC polypeptide.

The method described above is useful for identifying agents which may be useful in reducing spermatogenesis. An agent which reduces human sAC gene expression and is not cytotoxic is considered a possible agent for male contraceptive. Such agents are then further evaluated for safety and efficacy.

Screening of Candidate Agents Using Cell-Free Assays

Cell-free assays, *i.e.*, assays which measure human sAC polypeptide levels or function directly, include, but are not limited to, adenylyl cyclase activity assays, labeled *in vitro* protein-protein binding assays, protein-DNA binding assays, electrophoretic mobility shift assays, immunoassays for protein binding, and the like. Using these methods, one can identify substances that bind specifically to human sAC polypeptides. Such substances are useful as diagnostic agents to detect the presence of and/or to measure a level of human sAC polypeptide in a biological.

The screening assay can be a binding assay, wherein one or more of the molecules may be joined to a label, and the label directly or indirectly provide a detectable signal. Various labels include radioisotopes, fluorescers, chemilumescers, enzymes, specific binding molecules, particles, *e.g.* magnetic particles, and the like. Specific binding molecules include pairs, such as biotin and streptavidin, digoxin and antidigoxin *etc.* For the specific binding members, the complementary member would normally be labeled with a molecule that provides for detection, in accordance with known procedures.

A variety of other reagents may be included in the screening assays described herein. Where the assay is a binding assay, these include reagents like salts, neutral proteins, *e.g.* albumin, detergents, *etc.* that are used to facilitate optimal protein-protein binding, protein-DNA binding, and/or reduce non-specific or background interactions. Reagents that improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, *etc.* may be used. The components are added in any order that provides for the requisite binding. Incubations are performed at any suitable

temperature, typically between 4 and 40°C. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high-throughput screening. Typically between 0.1 and 1 hours will be sufficient.

Where the screening assay is a binding assay, one or more of the molecules may be joined to a label, where the label can directly or indirectly provide a detectable signal. Various labels include radioisotopes, fluorescers, chemilumescers, enzymes, specific binding molecules, particles, e.g. magnetic particles, and the like. Specific binding molecules include pairs, such as biotin and streptavidin, digoxin and antidigoxin etc. For the specific binding members, the complementary member would normally be labeled with a molecule that provides for detection, in accordance with known procedures.

The compounds having the desired activity (e.g., reduction of sAC mRNA levels, reduction of sAC polypeptide levels, or reduction of sAC adenylyl cyclase activity) may be administered in a physiologically acceptable carrier to a host for reversible reduction of spermatogenesis and reduction of male fertility. The agents may be administered in a variety of ways, orally, topically, parenterally e.g. subcutaneously, intraperitoneally, by viral infection, intravascularly, intramuscularly, by implantation (see, e.g., U.S. Patent No. 5,733,565), etc. Depending upon the manner of introduction, the compounds may be formulated in a variety of ways. The concentration of active compound in the formulation may vary from about 0.1-100 wt.%.

The pharmaceutical compositions can be prepared in various forms, such as granules, tablets, pills, suppositories, capsules, suspensions, salves, lotions and the like. Pharmaceutical grade organic or inorganic carriers and/or diluents suitable for oral and topical use can be used to make up compositions containing the therapeutically-active compounds. Diluents known to the art include aqueous media, vegetable and animal oils and fats. Stabilizing agents, wetting and emulsifying agents, salts for varying the osmotic pressure or buffers for securing an adequate pH value, and skin penetration enhancers can be used as auxiliary agents. Pharmaceutically acceptable excipients have been amply described in a variety of publications, including, for example, "Remington: The Science and Practice of Pharmacy", 19th Ed. (1995), or latest edition, Mack Publishing Co.

ISOLATED HUMAN sAC POLYNUCLEOTIDES

The present invention provides isolated human sAC polynucleotides. These isolated polynucleotides are useful for producing isolated polypeptides of the invention, as described below. They are also useful as hybridization probes for detecting the presence of nucleic acid molecules which hybridize under stringent hybridization conditions to an isolated polynucleotide of the invention. They are further useful in diagnostic methods, including methods to detect a human sAC mRNA in a biological sample, methods to identify polynucleotides having sequence similarity to human sAC

polynucleotides of the invention, methods to detect an alteration in a human sAC polynucleotide sequence in a cell, and methods to identify substances which modulate human sAC mRNA levels in a cell.

In some embodiments, the present invention provides isolated polynucleotides comprising the nucleotide sequence set forth in SEQ ID NO:1, or an identifying sequence thereof. An "identifying sequence" is a contiguous sequence of residues at least about 10 nucleotides (nt) to about 20 nt in length, usually at least about 50 nt to about 100 nt in length, that uniquely identifies the provided sequence. Encompassed in the term "human sAC polynucleotide" are isolated polynucleotides comprising at least about 10, at least about 20, at least about 50, at least about 66, at least about 100, at least about 200, at least about 300, at least about 500, at least about 1000, at least about 1500, at least about 2000, at least about 2500, at least about 3000, at least about 3500, at least about 4000, or at least about 4500 or more contiguous nucleotides of the sequence set forth in SEQ ID NO:1, up to the complete coding sequence. Other fragments of interest are those which encode an antigenic determinant, and which therefore can be used to elicit an immune response in a mammalian host.

As used herein, "human sAC polynucleotide" encompasses cDNA and genomic sequences. The term "cDNA" as used herein is intended to include all nucleic acids that share the arrangement of sequence elements found in native mature mRNA species, where sequence elements are exons and 3' and 5' non-coding regions. Normally mRNA species have contiguous exons, with the intervening introns, when present, removed by nuclear RNA splicing, to create a continuous open reading frame encoding the protein. The term "human sAC polynucleotide" further encompasses naturally occurring human polymorphisms.

A genomic sequence of interest comprises the nucleic acid present between the initiation codon and the stop codon, as defined in the listed sequences, including all of the introns that are normally present in a native chromosome. It may further include the 3' and 5' untranslated regions found in the mature mRNA. It may further include specific transcriptional and translational regulatory sequences, such as promoters, enhancers, etc., including about 1 kb, but possibly more, of flanking genomic DNA at either the 5' or 3' end of the transcribed region. The genomic DNA may be isolated as a fragment of 100 kbp or smaller; and substantially free of flanking chromosomal sequence. The genomic DNA flanking the coding region, either 3' or 5', or internal regulatory sequences as sometimes found in introns, contains sequences required for proper tissue and stage specific expression.

The sequence of the 5' flanking region may be utilized for promoter elements, including enhancer binding sites, that provide for developmental regulation in tissues where human sAC is expressed. The tissue specific expression is useful for determining the pattern of expression, and for providing promoters that mimic the native pattern of expression. Naturally occurring polymorphisms in the promoter region are useful for determining natural variations in expression, particularly those

that may be associated with, e.g., abnormal spermatid function, abnormal spermatogenesis, and/or abnormally low numbers of spermatids.

Alternatively, mutations may be introduced into the promoter region to determine the effect of altering expression in experimentally defined systems. Methods for the identification of specific DNA motifs involved in the binding of transcriptional factors are known in the art, e.g. sequence similarity to known binding motifs, gel retardation studies, etc. For examples, see Blackwell et al. (1995) *Mol. Med.* 1:194-205; Mortlock et al. (1996) *Genome Res.* 6:327-33; and Joulin and Richard-Foy (1995) *Eur. J. Biochem.* 232:620-626.

The regulatory sequences may be used to identify cis acting sequences required for transcriptional or translational regulation of expression, especially in different tissues or stages of development, and to identify cis acting sequences and trans-acting factors that regulate or mediate expression. Such transcription or translational control regions may be operably linked to one of the subject genes in order to promote expression of wild type or altered protein, or other proteins of interest in cultured cells, or in embryonic, fetal or adult tissues, and for gene therapy.

Isolated polynucleotides of the invention also include nucleic acids comprising sequences having sequence similarity or sequence identity to the sequence provided in SEQ ID NO:1. A previously identified cDNA sequence of rat sAC is found under GenBank Accession No. AF081941 (SEQ ID NO:3). The human and rat sAC coding regions share 84% nucleotide sequence identity when aligned, as shown in Figure 4. Isolated sAC polynucleotides of the invention share at least about 85%, more preferably at least about 90% or more, sequence identity with the sequence depicted in SEQ ID NO:1. Polynucleotides of the invention also include nucleic acids having sequence similarity or sequence identity to the sequences provided in SEQ ID NO:1. Nucleic acids having sequence similarity are detected by hybridization under low stringency conditions, for example, at 50°C and 10XSSC (0.9 M NaCl/0.09 M sodium citrate) and remain bound when subjected to washing at 55°C in 1XSSC.

Sequence identity can be determined by hybridization under stringent conditions, for example, at 50°C or higher and 0.1XSSC (9 mM NaCl/0.9 mM sodium citrate). Hybridization methods and conditions are well known in the art, see, e.g., U.S. Patent No. 5,707,829. Nucleic acids that are substantially identical to the provided nucleic acid sequences, e.g. allelic variants, genetically altered versions of the gene, etc., bind to the provided nucleic acid sequences (SEQ ID NO:1) under stringent hybridization conditions. By using probes, particularly labeled probes of DNA sequences, one can isolate homologous or related genes. The source of homologous genes can be any species, e.g. primate species, particularly human; rodents, such as rats and mice; canines, felines, bovines, ovines, equines, yeast, nematodes, etc.

"Hybridization" refers to the association of two nucleic acid sequences to one another by hydrogen bonding. Two sequences will be placed in contact with one another under conditions that favor hydrogen bonding. Factors that affect this bonding include: the type and volume of solvent; reaction temperature; time of hybridization; agitation; agents to block the non-specific attachment of the liquid phase sequence to the solid support (Denhardt's reagent or BLOTTO); concentration of the sequences; use of compounds to increase the rate of association of sequences (dextran sulfate or polyethylene glycol); and the stringency of the washing conditions following hybridization. See Sambrook, et al., supra (1989), Volume 2, chapter 9, pages 9.47 to 9.57.

"Stringency" refers to conditions in a hybridization reaction that favor association of very similar sequences over sequences that differ. For example, the combination of temperature and salt concentration should be chosen that is approximately 120 to 200°C below the calculated T_m of the hybrid under study. The temperature and salt conditions can often be determined empirically in preliminary experiments in which samples of genomic DNA immobilized on filters are hybridized to the sequence of interest and then washed under conditions of different stringencies. See Sambrook, et al., supra, at page 9.50.

Variables to consider when performing, for example, a Southern blot are (1) the complexity of the DNA being blotted and (2) the homology between the target and the sequences being detected. The total amount of the polynucleotides to be studied can vary a magnitude of 10, from 0.1 to 1 µg for a plasmid or phage digest to 10⁻⁹ to 10⁻⁸ µg for a single copy gene in a highly complex eukaryotic genome. For lower complexity polynucleotides, substantially shorter blotting, hybridization, and exposure times, a smaller amount of starting polynucleotides, and lower specific activity of a target polynucleotide can be used. For example, a single-copy yeast gene can be detected with an exposure time of only 1 hour starting with 1 µg of yeast DNA, blotting for two hours, and hybridizing for 4-8 hours with a target polynucleotide radiolabeled with 108 cpm/µg. For a single-copy mammalian gene a conservative approach would start with 10 µg of DNA, blot overnight, and hybridize overnight in the presence of 10% dextran sulfate using a target polynucleotide radiolabeled with greater than 108 cpm/µg, resulting in an exposure time of ~24 hours.

Several factors can affect the melting temperature (T_m) of a DNA-DNA hybrid between the target and sequence of interest, and consequently, the appropriate conditions for hybridization and washing. In many cases the target is not 100% homologous to the fragment. Other commonly encountered variables include the length and total G+C content of the hybridizing sequences and the ionic strength and formamide content of the hybridization buffer. The effects of all of these factors can be approximated by a single equation:

$$T_m = 81 + 16.6(\log 10Ci) + 0.4[\%G + C] - 0.6(\%formamide) - 600/n - 1.5(\%mismatch),$$

where C_i is the salt concentration (monovalent ions) and n is the length of the hybrid in base pairs (slightly modified from Meinkoth and Wahl, (1984) Anal. Biochem. 138: 267-284).

In designing a hybridization experiment, some factors affecting nucleic acid hybridization can be conveniently altered. The temperature of the hybridization and washes and the salt concentration during the washes are the simplest to adjust. As the temperature of the hybridization increases (i.e., stringency), it becomes less likely for hybridization to occur between strands that are nonhomologous, and as a result, background decreases. If the labeled probe is not completely homologous with the immobilized fragment (as is frequently the case in gene family and interspecies hybridization experiments), the hybridization temperature must be reduced, and background will increase. The temperature of the washes affects the intensity of the hybridizing band and the degree of background in a similar manner. The stringency of the washes is also increased with decreasing salt concentrations.

In general, convenient hybridization temperatures in the presence of 50% formamide are 42°C for a target polynucleotide with 95% to 100% sequence identity to the sequence to be detected, 37°C for 90% to 95% sequence identity, and 32°C for 85% to 90% sequence identity. For lower percentage sequence identity, formamide content should be lowered and temperature adjusted accordingly, using the equation above. If the homology between the target polynucleotide and the sequence to be detected are not known, the simplest approach is to start with both hybridization and wash conditions which are nonstringent. If nonspecific bands or high background are observed after autoradiography, the filter can be washed at high stringency and reexposed. If the time required for exposure makes this approach impractical, several hybridization and/or washing stringencies should be tested in parallel. Stringent conditions include hybridization in a solution of at least about 5 x SSC at 65°C, or at least about 4 x SSC at 42°C; see, for example, U.S. Patent No. 5,707,829, the disclosure of which is herein incorporated by reference.

Preferably, hybridization is performed using at least 18 contiguous nucleotides of SEQ ID NO:1. That is, when at least 18 contiguous nucleotides of SEQ ID NO:1 is used as a probe, the probe will preferentially hybridize with a nucleic acid or mRNA comprising the complementary sequence, allowing the identification and retrieval of the nucleic acids of the biological material that uniquely hybridize to the selected probe. Probes of more than 18 nucleotides can be used, e.g. probes of from about 25 nucleotides, about 50, about 75 nucleotides, to not more than about 100 nucleotides, but 18 nucleotides generally represents sufficient sequence for unique identification.

sAC mRNA is preferentially expressed in spermatids. The term "preferentially expressed in spermatids" indicates that sAC mRNA is expressed at a level generally at least about 2-fold, usually at least about 10-fold, typically at least about 20-fold or more higher in spermatids than in somatic cells of the body. A convenient reference cell is the Sertoli cell, which does not express sAC mRNA.

Whether an sAC mRNA is expressed preferentially in spermatids can be readily determined by those

skilled in the art, by determining the level of sAC mRNA in spermatids, in comparison with sAC mRNA levels in a somatic cell, including, but not limited to, Sertoli cells. Methods of measuring mRNA levels are known in the art, and any of these methods are suitable for use herein.

The nucleic acids of the invention also include naturally occurring variants of the nucleotide sequences, e.g. degenerate variants, allelic variants, etc. Variants of the nucleic acids of the invention are identified by hybridization of putative variants with nucleotide sequences disclosed herein, preferably by hybridization under stringent conditions. For example, by using appropriate wash conditions, variants of the nucleic acids of the invention can be identified where the allelic variant exhibits at most about 25-30% base pair mismatches relative to the selected nucleic acid probe. In general, allelic variants contain 15-25% base pair mismatches, and can contain as little as even 5-15%, or 2-5%, or 1-2% base pair mismatches, as well as a single base-pair mismatch.

The invention also encompasses homologs corresponding to the nucleic acids of SEQ ID NO:1, where the source of homologous genes can be any related species within the same genus or group. Within a group, homologs have substantial sequence similarity, e.g. at least about 80% sequence identity, usually at least 90%, more usually at least 95% between nucleotide sequences. Sequence similarity is calculated based on a reference sequence, which may be a subset of a larger sequence, such as a conserved motif, coding region, flanking region, etc. A reference sequence will usually be at least about 18 contiguous nt long, more usually at least about 30 nt long, and may extend to the complete sequence that is being compared. Algorithms for sequence analysis are known in the art, such as BLAST, described in Altschul et al. (1990) *J. Mol. Biol.* 215:403-10.

The nucleic acid compositions of the subject invention may encode all or a part of the subject polypeptides. SEQ ID NO:2 gives the amino acid translation of the nucleotide sequence given as SEQ ID NO:1. Accordingly, in some embodiments, an isolated polynucleotide of the invention comprises nucleotide sequences which encode a polypeptide comprising at least about 5, at least about 10, at least about 15, at least about 25, at least about 50, at least about 75, at least about 100, at least about 200, at least about 300, at least about 400, at least about 500, at least about 750, at least about 1000, at least about 1250, at least about 1500, or at least about 1600 or more contiguous amino acids of the sequence set forth in SEQ ID NO:2, up to the full length polypeptide. In some of these embodiments, an isolated polynucleotide of the invention comprises nucleotide sequences which encode a human sAC having the amino acid sequence depicted in SEQ ID NO:2. Also encompassed are human sAC polynucleotides encoding variants, fragments and fusion proteins of the aforementioned polypeptides. Accordingly, the invention provides a human sAC polynucleotide which encodes a variants of a human sAC polypeptide, including variants having conservative amino acid substitutions, and fragments thereof; and fusion proteins comprising any one of the aforementioned polypeptides and a heterologous

polypeptide (i.e., a non-human sAC polypeptide, other than the rat sAC polypeptide sequence provided under GenBank accession No. A081941).

Double or single stranded fragments may be obtained of the DNA sequence by chemically synthesizing oligonucleotides in accordance with conventional methods, by restriction enzyme digestion, by polymerase chain reaction (PCR) amplification, etc. For the most part, DNA fragments will be of at least 18 nt, usually at least 25 nt or 50 nt, and may be at least about 100 nt. Such small DNA fragments are useful as primers for PCR, hybridization screening probes, etc. Larger DNA fragments, i.e. greater than 500 nt are useful for production of the encoded polypeptide. For use in amplification reactions, such as PCR, a pair of primers will be used. The exact composition of the primer sequences is not critical to the invention, but for most applications the primers will hybridize to the subject sequence under stringent conditions, as known in the art. It is preferable to choose a pair of primers that will generate an amplification product of at least about 50 nt, preferably at least about 100 nt. Algorithms for the selection of primer sequences are generally known, and are available in commercial software packages. Amplification primers hybridize to complementary strands of DNA, and will prime towards each other.

Also encompassed by the invention are polynucleotides complementary to a human sAC polynucleotide, as defined above. Further encompassed are human sAC antisense polynucleotides and ribozymes. Various derivatives of the antisense sequence may be prepared, where the phosphates may be modified, where oxygens may be substituted with sulfur and nitrogen, the sugars may be modified, and the like. The antisense sequences may be used by themselves or in conjunction with various toxic moieties, such as metal chelates, sensitizers, ribozymes, and the like. Antisense and/or ribozyme sequences may be used to inhibit spermatogenesis. Antisense polynucleotides, and methods of using such, are described in numerous publications, including, e.g., "Antisense Technology: A Practical Approach" Lichtenstein and Nellen, eds. (1997) IRL Press.

Polynucleotides of the invention are isolated and obtained in substantial purity, generally as other than an intact chromosome. Usually, the DNA will be obtained substantially free of other nucleic acid sequences, generally being at least about 50%, usually at least about 90% pure and are typically "recombinant", i.e. flanked by one or more nucleotides with which it is not normally associated on a naturally occurring chromosome.

The DNA sequences are used in a variety of ways. They can be used in methods to detect human sAC mRNA in a biological sample, as described in more detail below.

The human sAC-encoding DNA may also be used to identify expression of the gene in a biological specimen. The manner in which one probes cells for the presence of particular nucleotide sequences, as genomic DNA or RNA, is well established in the literature and does not require elaboration here. mRNA may be isolated from a cell sample, or may be detected without being first

isolated. mRNA may be amplified by RT-PCR, using reverse transcriptase to form a complementary DNA (cDNA) strand, followed by polymerase chain reaction amplification using primers specific for the subject DNA sequences. Alternatively, mRNA sample is separated by gel electrophoresis, transferred to a suitable support, *e.g.* nitrocellulose, nylon, *etc.*, and then probed with a fragment of the subject DNA as a probe. Other techniques, such as oligonucleotide ligation assays, *in situ* hybridizations, and hybridization to DNA probes arrayed on a solid chip may also find use. Detection of mRNA hybridizing to a human sAC sequence is indicative of human sAC gene expression in the sample.

The human sAC nucleic acid sequence may be modified for a number of purposes, particularly where they will be used intracellularly, for example, by being joined to a nucleic acid cleaving agent, *e.g.* a chelated metal ion, such as iron or chromium for cleavage of the gene; or the like.

The sequence of the human sAC locus, including flanking promoter regions and coding regions, may be mutated in various ways known in the art to generate targeted changes in promoter strength, sequence of the encoded protein, *etc.* The DNA sequence or product of such a mutation will be substantially similar to the sequences provided herein, *i.e.* will differ by at least one nucleotide or amino acid, respectively, and may differ by at least two but generally not more than about ten nucleotides or amino acids. The sequence changes may be substitutions, insertions or deletions. Deletions may further include larger changes, such as deletions of a domain or exon. Other modifications of interest include epitope tagging, *e.g.* with the FLAG system, HA, *etc.* For studies of subcellular localization, fusion proteins with green fluorescent proteins (GFP) may be used. Such mutated genes may be used to study structure-function relationships of human sAC polypeptides with other polypeptides, or to alter properties of the proteins that affect their function or regulation. Such modified human sAC sequences can be used, for example, to generate transgenic animals.

Techniques for *in vitro* mutagenesis of cloned genes are known. Examples of protocols for scanning mutations may be found in Gustin et al., 1993 *Biotechniques* 14:22 ; Barany, 1985 *Gene* 37:111-23; Colicelli et al., 1985 *Mol Gen Genet* 199:537-9; and Prentki et al., 1984 *Gene* 29:303-13. Methods for site-specific mutagenesis can be found in Sambrook et al., 1989 *Molecular Cloning: A Laboratory Manual*, CSH Press, pp. 15.3-15.108; Weiner et al., 1993 *Gene* 126:35-41; Sayers et al., 1992 *Biotechniques* 13:592-6; Jones and Winistorfer, 1992 *Biotechniques* 12:528-30; Barton et al., 1990 *Nucleic Acids Res.* 18:7349-55; Marotti and Tomich, 1989 *Gene Anal. Tech.* 6:67-70; and Zhu 1989 *Anal. Biochem.* 177:120-4.

Human sAC polynucleotides can be obtained by chemical or biochemical synthesis, by recombinant DNA techniques, or by isolating the nucleic acids from a biological source. For example, the nucleic acid may be synthesized using solid phase synthesis techniques, as are known in the art.

Oligonucleotide synthesis is also described in Edge et al., Nature (1981) 292:756; Duckworth et al., Nucleic Acids Res. (1981) 9:1691 and Beaucage & Caruthers, Tet. Letts (1981) 22: 1859. Following preparation of the nucleic acid, the nucleic acid is then ligated to other members of the expression system to produce an expression cassette or system comprising a nucleic acid encoding the subject product in operational combination with transcriptional initiation and termination regions, which provide for expression of the nucleic acid into the subject polypeptide products under suitable conditions.

ISOLATED HUMAN SOLUBLE ADENYLYL CYCLASE POLYPEPTIDES

The present invention provides isolated human sAC polypeptides. The polypeptides are useful for a variety of purposes, including use in methods of identifying factors that bind to human sAC polypeptides; generating antibodies specific for human sAC polypeptides; and use in methods of identifying substances which modulate human sAC enzyme activity.

The term "human sAC polypeptide" encompasses an amino acid sequence encoded by an open reading frame (ORF) of the human sAC polynucleotides described herein, including the full-length native polypeptide and fragments thereof, particularly biologically active fragments and/or fragments corresponding to functional domains, e.g. a region or domain having adenylyl cyclase activity, etc.; antigenic fragments thereof; and including fusions of the subject polypeptides to other proteins or parts thereof.

As used herein, "human sAC polypeptide" refers to an amino acid sequence of a recombinant or non-recombinant polypeptide having an amino acid sequence of i) a native human sAC polypeptide, ii) a fragment of a human sAC polypeptide, iii) polypeptide analogs of a human sAC polypeptide, iv) variants of a human sAC polypeptide; v) an immunologically active fragment of a human sAC polypeptide; and vi) fusion proteins comprising a human sAC polypeptide. Human sAC polypeptides of the invention can be obtained from a human biological sample, or from any source whether natural, synthetic, semi-synthetic or recombinant. "Human sAC polypeptide" refers to the amino acid sequences of isolated human sAC polypeptide obtained from a human, and is meant to include all naturally-occurring allelic variants, and is not meant to limit the amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule.

Those skilled in the art will appreciate that changes can be made to the sequences depicted in SEQ ID NO:2 without substantially affecting a function of the human sAC polypeptide. Thus, the term "human sAC polypeptide" encompasses polypeptides with conservative amino acid substitutions compared with the sequences depicted in SEQ ID NO:2. Examples of conservative amino acid substitutions include Ser/Thr; Ala/Val; Leu/Ile; Asp/Glu; and Phe/Tyr. Clearly, other amino acid substitutions, deletions, and insertions can be made to the polypeptide without affecting one or more

functions of the polypeptide. Those skilled in the art, given the guidance provided in the instant specification, can readily determine whether a given function of a human sAC polypeptide is preserved. One such function is adenylyl cyclase activity. Accordingly, in some embodiments of the invention, an isolated human sAC polypeptide has adenylyl cyclase activity.

5 Whether a human sAC polypeptide has adenylyl cyclase activity can be readily determined by those skilled in the art. Any known method to assay adenylyl cyclase activity can be used, as described above. Adenylyl cyclase activity can be measured in cell lysates comprising sAC, using isolated sAC, or in intact cells comprising sAC, as described above.

10 The term "human sAC polypeptide" encompasses a polypeptide comprising from at least about 5, at least about 10, at least about 15, at least about 25, at least about 43, at least about 50, at least about 75, at least about 100, at least about 200, at least about 300, at least about 400, at least about 500, at least about 750, at least about 1000, at least about 1250, at least about 1500, or at least about 1600 or more contiguous amino acids of the sequence set forth in SEQ ID NO:2. In some embodiments, a human sAC polypeptide comprises the entire sequence set forth in SEQ ID NO:2.

15 The human sAC and rat sAC amino acid sequences share about 76.7% amino acid sequence identity, as measured using the BLASTP program 2.0.9 (matrix blosum62; gap open = 9; gap extension = 2; dropoff = 50; wordsize = 3; no filter). Accordingly, also encompassed by the term "human sAC polypeptide" is a polypeptide sharing at least about 77%, more preferably at least about 80 %, even more preferably at least about 85%, even more preferably at least about 90%, even more preferably at least about 95% or more amino acid sequence identity with the sequence depicted in SEQ ID NO:2.

20 A human sAC polypeptide need not be full length in order to retain adenylyl cyclase activity. Accordingly, in some embodiments, a human sAC polypeptide has a C-terminal truncation or internal deletion(s), while retaining adenylyl cyclase activity. Thus, a human sAC polypeptide may comprise, or may consist essentially of, from amino acid 1 to about amino acid 500, from amino acid 1 to about amino acid 490, from amino acid 1 to about amino acid 480, or from amino acid 1 to about amino acid 470, of the sequence provided in SEQ ID NO:2. In one embodiment, a human sAC polypeptide consists essentially of from amino acid 1 to amino acid 469 of the sequence provided in SEQ ID NO:2.

30 Production of isolated human sAC polypeptides

Isolated human sAC polypeptides of the invention can be obtained by any known method, or a combination of such methods, including isolation from natural sources; production by chemical synthesis; and production by standard recombinant techniques.

35 Human sAC polypeptides can be isolated from a biological source, using affinity chromatography, e.g., using antibodies specific for human sAC are immobilized on a solid support.

Alternatively, human sAC polypeptides can be isolated from a human biological source using a combination of anion exchange, and size exclusion chromatography, using a protocol such as that described in Buck et al. (1999) *Proc. Natl. Acad. Sci. USA* 96:79-84.

The polypeptides may be expressed in prokaryotes or eukaryotes in accordance with conventional ways, depending upon the purpose for expression. For large scale production of the protein, a unicellular organism, such as *E. coli*, *B. subtilis*, *S. cerevisiae*, insect cells in combination with baculovirus vectors, or cells of a higher organism such as vertebrates, particularly mammals, e.g. COS 7 cells, CHO cells, HEK293 cells, MA-10 cells, and the like, may be used as the expression host cells. In some situations, it is desirable to express the gene in eukaryotic cells, where the protein will benefit from native folding and post-translational modifications. The polypeptide can then be isolated from cell culture supernatant or from cell lysates using affinity chromatography methods or anion exchange/size exclusion chromatography methods, as described above.

With the availability of the protein or fragments thereof in large amounts, by employing an expression host, the protein may be isolated and purified in accordance with conventional ways. A lysate may be prepared of the expression host and the lysate purified using HPLC, exclusion chromatography, gel electrophoresis, affinity chromatography, or other purification technique.

RECOMBINANT VECTORS

The present invention further provides recombinant vectors ("constructs") comprising a polynucleotide of the invention. Recombinant vectors include vectors used for propagation of a polynucleotide of the invention, and expression vectors. The present invention further provides recombinant vectors comprising human sAC polynucleotides of the invention. Recombinant vectors are useful for propagation of the subject human sAC polynucleotides (cloning vectors). They are also useful for effecting expression of a human sAC polynucleotide in a cell. The choice of appropriate vector is well within the skill of the art. Many such vectors are available commercially.

The subject nucleic acid molecules are generally propagated by placing the molecule in a vector. Viral and non-viral vectors are used, including plasmids. The choice of plasmid will depend on the type of cell in which propagation is desired and the purpose of propagation. Certain vectors are useful for amplifying and making large amounts of the desired DNA sequence.

Other vectors are suitable for expression in cells in culture. These vectors will generally include regulatory sequences ("control sequences" or "control regions") which are necessary to effect the expression of a human sAC polynucleotide to which they are operably linked. Still other vectors are suitable for transfer and expression in cells in a whole organism or person.

Expression vectors generally have convenient restriction sites located near the promoter sequence to provide for the insertion of nucleic acid sequences encoding heterologous proteins. A

selectable marker operative in the expression host may be present. Expression vectors may be used for the production of fusion proteins, where the exogenous fusion peptide provides additional functionality, i.e. increased protein synthesis, stability, reactivity with defined antisera, an enzyme marker, e.g. β -galactosidase, etc.

5 Expression cassettes may be prepared comprising a transcription initiation region, the gene or fragment thereof, and a transcriptional termination region. Of particular interest is the use of sequences that allow for the expression of functional epitopes or domains, usually at least about 8 amino acids in length, more usually at least about 15 amino acids in length, at least about 25 amino acids, at least about 45 amino acids, and up to the complete open reading frame of the gene. After
10 introduction of the DNA, the cells containing the construct may be selected by means of a selectable marker, the cells expanded and then used for expression.

The expression cassettes may be introduced into a variety of vectors, e.g. plasmid, BAC, YAC, bacteriophage such as lambda, P1, M13, etc., animal or plant viruses, and the like, where the vectors are normally characterized by the ability to provide selection of cells comprising the expression
15 vectors. The vectors may provide for extrachromosomal maintenance, particularly as plasmids or viruses, or for integration into the host chromosome. Where extrachromosomal maintenance is desired, an origin sequence is provided for the replication of the plasmid, which may be low- or high copy-number. A wide variety of markers are available for selection, particularly those which protect against toxins, more particularly against antibiotics. The particular marker that is chosen is selected in
20 accordance with the nature of the host, where in some cases, complementation may be employed with auxotrophic hosts. Introduction of the DNA construct may use any convenient method, e.g. conjugation, bacterial transformation, calcium-precipitated DNA, electroporation, fusion, transfection, infection with viral vectors, biolistics, etc.

25 HOST CELLS

The present invention further provides host cells, which may be isolated host cells, comprising human sAC polynucleotides of the invention. Suitable host cells include prokaryotes such as *E. coli*, *B. subtilis*, eukaryotes, including insect cells in combination with baculovirus vectors, yeast cells, such as *Saccharomyces cerevisiae*, or cells of a higher organism such as vertebrates, including amphibians
30 (e.g., *Xenopus laevis* oocytes), and mammals, particularly mammals, e.g. COS cells, CHO cells, HEK293 cells, MA-10 cells, and the like, may be used as the expression host cells. Host cells can be used for the purposes of propagating a human sAC polynucleotide, for production of a human sAC polypeptide, or in cell-based methods for identifying agents which modulate a level of sAC mRNA and/or protein and/or enzyme activity in a cell.

The subject nucleic acids can be used to generate transgenic animals or site specific gene modifications in cell lines. The modified cells or animals are useful in the study of sAC function and regulation. For example, a series of small deletions or substitutions may be made in the sAC gene to determine the role of different coding regions in spermatogenesis, signal transduction, substrate binding, *etc.*

DNA constructs for homologous recombination will comprise at least a portion of the sAC gene with the desired genetic modification, and will include regions of homology to the target locus. Conveniently, markers for positive and negative selection are included. Methods for generating cells having targeted gene modifications through homologous recombination are known in the art. For various techniques for transfecting mammalian cells, see Keown *et al.* (1990) Methods in Enzymology 185:527-537.

For embryonic stem (ES) cells, an ES cell line may be employed, or ES cells may be obtained freshly from a host, *e.g.* mouse, rat, guinea pig, *etc.* Such cells are grown on an appropriate fibroblast-feeder layer or grown in the presence of leukemia inhibiting factor (LIF). When ES cells have been transformed, they may be used to produce transgenic animals. After transformation, the cells are plated onto a feeder layer in an appropriate medium. Cells containing the construct may be detected by employing a selective medium. After sufficient time for colonies to grow, they are picked and analyzed for the occurrence of homologous recombination. Those colonies that show homologous recombination may then be used for embryo manipulation and blastocyst injection. Blastocysts are obtained from 4 to 6 week old superovulated females. The ES cells are trypsinized, and the modified cells are injected into the blastocoel of the blastocyst. After injection, the blastocysts are returned to each uterine horn of pseudopregnant females. Females are then allowed to go to term and the resulting litters screened for mutant cells having the construct. By providing for a different phenotype of the blastocyst and the ES cells, chimeric progeny can be readily detected. The chimeric animals are screened for the presence of the sAC gene and males and females having the modification are mated to produce homozygous progeny. The transgenic animals may be any non-human mammal, such as laboratory animals, domestic animals, *etc.* The transgenic animals may be used to determine the effect of a candidate drug on spermatogenesis in an *in vivo* environment.

ANTIBODIES

The present invention provides antibodies, which may be isolated antibodies, specific for human sAC polypeptides of the invention. Such antibodies are useful, for example, in methods of detecting the presence of a human sAC polypeptide in a biological sample, and in methods of isolating a human sAC polypeptide from a biological sample.

Isolated human sAC polypeptides of the invention are useful for the production of antibodies, where short fragments provide for antibodies specific for the particular polypeptide, and larger fragments or the entire protein allow for the production of antibodies over the surface of the polypeptide. Accordingly, the invention provides isolated antibodies which specifically bind a human sAC polypeptide, or antigenic fragment thereof. Antibodies may be raised to the wild-type or variant forms. Antibodies may be raised to isolated peptides corresponding to these domains, or to the native protein. Antibodies may be raised to polypeptides and/or peptide fragments of sAC from other species. These antibodies may cross-react with human sAC polypeptides. For example, antibodies can be raised using as an immunogen a rat sAC peptide fragment, e.g., amino acids 1518-1607, amino acids 569-593, and/or amino acids 92-108 of the rat sAC amino acid sequence depicted in Figure 2.

The human sAC polypeptides of the invention are useful for the production of antibodies, where short fragments provide for antibodies specific for the particular polypeptide, and larger fragments or the entire protein allow for the production of antibodies over the surface of the polypeptide. As used herein, the term "antibodies" includes antibodies of any isotype, fragments of antibodies which retain specific binding to antigen, including, but not limited to, Fab, Fv, scFv, and Fd fragments, fusion proteins comprising such antibody fragments, and chimeric antibodies. "Antibody specificity", in the context of antibody-antigen interactions, is a term well understood in the art, and indicates that a given antibody binds to a given antigen, wherein the binding can be inhibited by that antigen or an epitope thereof which is recognized by the antibody, and does not substantially bind to unrelated antigens. Methods of determining specific antibody binding are well known to those skilled in the art, and can be used to determine the specificity of antibodies of the invention for a human sAC polypeptide.

Antibodies are prepared in accordance with conventional ways, where the expressed polypeptide or protein is used as an immunogen, by itself or conjugated to known immunogenic carriers, e.g. KLH, pre-S HBsAg, other viral or eukaryotic proteins, or the like. Various adjuvants may be employed, with a series of injections, as appropriate. For monoclonal antibodies, after one or more booster injections, the spleen is isolated, the lymphocytes immortalized by cell fusion, and then screened for high affinity antibody binding. The immortalized cells, i.e. hybridomas, producing the desired antibodies may then be expanded. For further description, see *Monoclonal Antibodies: A Laboratory Manual*, Harlow and Lane eds., Cold Spring Harbor Laboratories, Cold Spring Harbor, New York, 1988. If desired, the mRNA encoding the heavy and light chains may be isolated and mutagenized by cloning in *E. coli*, and the heavy and light chains mixed to further enhance the affinity of the antibody. Alternatives to *in vivo* immunization as a method of raising antibodies include binding to phage display libraries, usually in conjunction with *in vitro* affinity maturation.

COMPOSITIONS

The present invention further provides compositions, including pharmaceutical compositions, comprising the polypeptides, polynucleotides, recombinant vectors, host cells, and antibodies of the invention. These compositions may include a buffer, which is selected according to the desired use of the polypeptide, polynucleotide, recombinant vector, host cell, or antibody, and may also include other substances appropriate to the intended use. Those skilled in the art can readily select an appropriate buffer, a wide variety of which are known in the art, suitable for an intended use. In some instances, the composition can comprise a pharmaceutically acceptable excipient, a variety of which are known in the art and need not be discussed in detail herein. Pharmaceutically acceptable excipients have been amply described in a variety of publications, including, for example, "Remington: The Science and Practice of Pharmacy", Alfonso R. Gennaro (latest edition), Lippincott, Williams, and Wilkins.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celcius, and pressure is at or near atmospheric.

EXAMPLE 1

Genomic structure of the human soluble adenylyl cyclase gene

A human testis library was screened with a rat cDNA sequence corresponding to the catalytic and regulatory domains of sAC. Five positive clones were isolated. Two PAC clones from human chromosome 1q24 were sequenced in their entirety. The human sAC gene comprises 32 exons distributed over 120 kb of genomic DNA. The 4896-nucleotide sequence encoding human sAC is shown in Figure 3, and is set forth in SEQ ID NO:1. An alignment of human and rat (GenBank Accession No. AF081941; SEQ ID NO:3) sAC-encoding nucleotide sequence, provided in Figure 4, shows that these two sequences share 84% nucleotide sequence identity.

The human sAC amino acid sequence is depicted in Figure 1. The C1 domain is encoded by exons 2-6, and the C2 domain is encoded by exons 8-11. The encoded human sAC polypeptide has 1614 amino acids (SEQ ID NO:2). An alignment of the 1608-amino acid rat and 1614-amino acid human amino sequences is shown in Figure 2. The human sAC and rat sAC amino acid sequences

were aligned using the BLASTP program 2.0.9 (matrix file: blosum50; gap open = 9; gap extension = 2; dropoff = 50; wordsize = 3; no filter). Using these parameters, it was determined that the human sAC amino acid sequence shares 76.7% amino acid sequence identity with the rat sAC amino acid sequence (SEQ ID NO:4), as shown in Figure 2. The C1 and the C2 domains, together with exon 13, which encodes the P loop, are the most highly conserved regions between rat and human sAC. The C-terminal half of human sAC protein is less conserved, with the exception of the leucine zipper domain, and a region homologous to a tetratricopeptide repeat, a 34-amino acid domain involved in protein-protein interactions.

EXAMPLE 2

Bicarbonate ions activate sAC adenylyl cyclase activity

Experiments were conducted to determine whether bicarbonate ions affect sAC adenylyl cyclase activity. The results are shown in Figure 5. HEK-293 cells were transfected with either 20 μ g of empty plasmid ("mock transfected cells"), C1C2-recombinant human sAC ("C1-C2 Rec. Hum sAC"), or full-length human sAC (FL Rec. Hum sAC") constructs. After harvesting and homogenization of the cells, the 100,000 x g supernatant was used for the assay. Adenylate cyclase activity was measured in the presence (solid bars) or absence (stippled bars) of 50 mM bicarbonate. Supernatants extracted from human sperm were used as a source of native human sAC ("Human Sperm AC"). Data are expressed as picomoles of cAMP formed per minute per milligram of total cellular protein. The data shown in Figure 5 demonstrate that human sAC adenylyl cyclase activity is activated by bicarbonate.

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

What is claimed is:

1. An isolated polypeptide comprising at least about 43 contiguous amino acids of the
5 sequence depicted in SEQ ID NO:2.

2. The isolated polypeptide of claim 1, wherein said polypeptide has an amino acid
sequence at least about 78% identical to the amino acid sequence depicted in SEQ ID NO:2.

10 3. The isolated polypeptide of claim 1, wherein said polypeptide has the amino acid
sequence depicted in SEQ ID NO:2

4. The isolated polypeptide of claim 1, wherein said polypeptide has manganese-
dependent soluble adenyl cyclase activity.

15 5. A composition comprising the polypeptide of claim 1, and a buffer.

6. An isolated polynucleotide which encodes a polypeptide comprising at least about 6
contiguous amino acids of the sequence depicted in SEQ ID NO:2.

20 7. The isolated polynucleotide of claim 6, wherein said polynucleotide encodes a
polypeptide having the sequence depicted in SEQ ID NO:2.

8. The isolated polynucleotide of claim 6, wherein said polynucleotide has a nucleotide
25 sequence at least about 85% identical to the sequence depicted in SEQ ID NO:1.

9. The isolated polynucleotide of claim 8, wherein said polynucleotide hybridizes,
under stringent conditions, with the polynucleotide sequence depicted in SEQ ID NO:1.

30 10. The isolated polynucleotide of claim 9, wherein said polynucleotide has the
nucleotide sequence depicted in SEQ ID NO:1.

11. An isolated polynucleotide comprising at least about 66 contiguous nucleotides of
the sequence set forth in SEQ ID NO:1.

12. The isolated polynucleotide of claim 11, wherein the polynucleotide encodes a manganese-dependent soluble human adenylyl cyclase.

13. A recombinant vector comprising the polynucleotide of claim 6.

14. An isolated host cell comprising the polynucleotide of claim 13.

15. The isolated host cell of claim 14, wherein the cell is a prokaryotic cell.

16. The isolated host cell of claim 14, wherein the cell is a eukaryotic cell.

17. An antibody which specifically binds a polypeptide comprising at least about 6 contiguous amino acids of the sequence set forth in SEQ ID NO:2.

18. The antibody of claim 17, wherein said antibody is attached to a detectable label.

19. The antibody of claim 17, wherein said antibody is attached to a solid support.

20. A method of identifying a substance which modulates human soluble adenylyl cyclase activity, comprising the steps of:

(a) contacting human soluble adenylyl cyclase with a substance to be tested, forming a test sample;

(b) measuring adenylyl cyclase activity in the test sample, wherein an effect on adenylyl cyclase activity in the test sample compared to a control sample in the absence of the substance being tested is an indication that the substance modulates adenylyl cyclase activity.

21. The method of claim 20, wherein said adenylyl cyclase is present in a cell lysate.

22. The method of claim 20, wherein said adenylyl cyclase is present in an intact cell, and wherein said adenylyl cyclase is encoded by nucleotide sequences comprised within an exogenous expression vector.

23. A method of inhibiting spermatogenesis in a male, comprising administering to the male a substance which preferentially inhibits soluble adenylyl cyclase activity.

24. The method of claim 23, wherein the male is a human.

25. The method of claim 24, wherein the male is a rodent.

5 26. A reversible method of male contraception, comprising administering to a male a pharmaceutical composition comprising an effective amount of a substance which preferentially inhibits soluble adenylyl cyclase.

27. The method of claim 26, wherein the male is a human male.

10

28. The method of claim 26, wherein the male is a rodent.

29. A method of reducing the number of motile sperm produced by a male, comprising administering to a male a pharmaceutical composition comprising an effective amount of a substance
15 which preferentially inhibits soluble adenylyl cyclase.

1/10

FIG. 1

Human sAC deduced amino acid sequence

MNTPKEEFQDWPIVRIAHLPLDIVYGHFSPERPFMDYFDGVLMFVDISGFTAMTEKFSSAMYMDRGAE
QLVEILNYHISAIVEKVLIFGGDILKFAGDALLALWRVERKQLKNIITVVIKCSLEIHGLFETQEWEEG
LDIRVKIGLAAGHISMLVFGDETHSHFLVIGQAVDDVRLAQNMAQMNDVILSPNCWQLCDRSMIEIESV
PDQRAVKVNFLKPPPNFNFDEFFTKCTTFMHYYPSGEHKNLLRLACTLKPDPELEMSLQKYVMESILKQ
IDNKQLQGYLSELRPVTIVFVNLMFEDQDKAEIGPAIQDAYMHITSVLKIFQQQINKVFMFDKGCSTL
CVFGFPGKEKVPDELTHALECAMDIFDFCSQVHKIQTVSIGVASGIVFCGIVGHTVRHEYTVIGQKVNLA
ARMMYYPGIIVTCDSTYNGSNLPAYFFKELPKKVMKGVADSGPLYQYWGRTKVMFGMACLICNRKED
YPLLGRNKEINYFMYTMKKFLISNSSQVLMYEGLPGYKGSQILMKIEYLAQGKNHRIIAISLNKISFHQ
TFYTIQMFMANVLGLDTCYKERQTNLNRKVMTLLEDEKFYCLLNDIFHVQFPISREISRMSTLKKQKQ
LEILFMKILKLVKEERIIFIIDEAQVDSTSWRFMEKLIRTLPIFIIMSLCPFVNIPCAAAARAVIKNR
NTTYIVVGAVQPNDISNKICLDLNVSCISKELDSYLGECSGIPFYCEELLKNLEHHEVLVFQQTSEEE
KTNRTWNNLFKYSIKLTEKLNMTLHSDKESEEVCHLTSGVRLKNLSPPTSLKEISLIQLDSMRLSHQM
LVRCAAIIGLTFTTELLFEILPCWNMKNMKTATLVESNIFYCFRNGKELQKALKQNDPSFEVHYRSL
SLKPSEGMDHGEELQRELENEVIECHIRFCNPMQKTAYELWLKDQRKAMHLKCARFLEEDAHRCDH
CRGRDFIPYHHFTVNIRLNALDMDAIIKMMAMSHGFKTEELILSNSEIPETSAFFPENRSPEEIREKIL
NFFDHVLTMMKTSDEDIIPLESCQCEEILEIVILPLAHHFLALGENDKALYYFLEIASAYLIFCDNYMA
YMYLNEGQKLLKTLKDKSWSQTFESATFYSLKGEVCFNMGQIVLAKKMLRKALKLLNRIFPYNLISLF
LHIHVEKNRHFHYVNRQAQESPPPGKKRLAQLYRQTVCLSLWRIYSYSYLFHCKYYAHLAVMMQMNTA
LETQNCFQIIKAYLDYSLYHLAGYKGVWFKEYVMAMEHIFNLPLKGEIEIVAYVAETLVFNKLIMGH
LDLAIELGSRALQMWALLQPNRHYQSLCRLSRCLLLNSRYPQLIQVLGRLWELSVTQEHIFSKAFFYF
VCLDILLYSGFVYRTFEECLEFIHQYENNRILKFHSGLLLGLYSSVAIWYARLQEWDFYKFSNRAKNL
LPRRTMTLTYYDGISRYMEGQVLHLQKQIKEQSENAQASGEELLKNLENLVAQNTTGPVFCPRLYHLMA
YVCILMGDGQKCGFLNTALRLSETQGNILEKCWLNMNKESWYSTSELKEDQWLQTIILSLPSWEKIVAG
RVNIQDLQKNKFLMRANTVDNHF

2/10

FIG. 2A

Alignment of human and rat sAC amino acid sequences

rsAC	1	MSARRQELQDRAIVKIAAHLPLDIVYGDSPERPSSVKCFDGLVMFVDISGFTAMTEKFSIAMYMDRGAEQ
hsAC	1	MNTPKKEFDQWPIVRIAAHLPLDIVYGHSPERPFDYFDGLVMFVDISGFTAMTEKFSIAMYMDRGAEQ
rsAC	71	LVEILNYYISAIVEKVLIFGGDILKFAGDALLALWVERKQLKNIITVVIKCSLEIHGLFEAKEVEEGLD
hsAC	71	LVEILNYYISAIVEKVLIFGGDILKFAGDALLALWVERKQLKNIITVVIKCSLEIHGLFETQEWEEGLD
rsAC	141	IRVKIGLAAGHITMLVFGDETRNYFLVIGQAVDDVRLAQNMAQMNDVILSPNCWQLCDRSMIEIERIPDQ
hsAC	141	IRVKIGLAAGHISMLVFGDETHSHFLVIGQAVDDVRLAQNMAQMNDVILSPNCWQLCDRSMIEIESVPDQ
rsAC	211	RAVKVSFLKPPPTFNFDEFFAKCMFMDYYPGSDHKNFRLACMLESDPELELSLQKYVMEIILKQIDDK
hsAC	211	RAVKVNFLLKPPNFNFDEFFTKCTFMHYYPGSEHKNFRLACTLKPDPELEMSLQKYVMESILKQIDNK
rsAC	281	QLRGYLSELRPVTIVFVNLMFKEQDKAEVIGSAIQAAQVHITSVLKVFRRGQINKVFMFDKGCSTLCVFGF
hsAC	281	QLQGYLSELRPVTIVFVNLMFEDQDKAEVIGPAIQDAYMHITSVLKIFQGGQINKVFMFDKGCSTLCVFGF
rsAC	351	PGEKAPDEIITHALESADVIFDFCSQVHKIRTVSIGVASGIVFCGIVGHTVRHEYTVIGQKVNIAARMMMY
hsAC	351	PGEKVPDELTHALECAMDIFDFCSQVHKIQTVSIGVASGIVFCGIVGHTVRHEYTVIGQKVNLAARMMMY
rsAC	421	YPGIVTCDSVTYDGSNLPAYFFKELPKKVMKGVADEPGVYQCLQNEKVMFGMAYLICNRYEGYPLLGRV
hsAC	421	YPGIVTCDSVTYNGSNLPAYFFKELPKKVMKGVADESGPLYQYWRTEKVMFGMAQLICNRKEDYPLLGRN
rsAC	491	REIDYFMSTMKDFLMTNCSRVLMEYGLPGYGKSQVLMIEYLAHQENHRAVAIALTKISFHQNFYTIQI
hsAC	491	KEINYFMYTMKFLISNSSQVLMYGLPGYGKSQILMKIEYLAQGNHRIIAISLNKISFHQTFYTIQM
rsAC	561	LMANVLGLDTCKHYKERQTNLQNRVKTLLDQYHCLLNDIFHVQFPVSREMSRMSKIRKQKQLEALFMKI
hsAC	560	FMANVLGLDTCKHYKERQTNLRNKVMTLLDEKFYCLLNDIFHVQFPISREISRMSTLKKQKQLEILFMKI
rsAC	631	LEQTVREERIIFIIDEAQVDVASWAFIEKLIRSMPIFIVMSLCPFPETPCAAANAIMKNRNTTYITIGT
hsAC	630	LKLIVKEERIIFIIDEAQVDSTSWRFMEKLIRTLPIFIVMSLCPFVNIPCAAARAVIKNRNTTYIVMGA
rsAC	701	MQPQETIRDKVQVDLSVSSIPRELDSTLVGSCGIPYCEELLKNLDHHRILIFQQAEEKTNVTWNNLF
hsAC	700	MQPNDSIKIQLDLNVSCISKELDSYLVGSCGIPYCEELLKNLEHHEMLMFQQTSEKTNRTWNNLF
rsAC	771	KYSVKPTEDMYLYTSIAAGQ-KEACYLTSGVRLKNLSPPASLKEISLMQLDSMSLSHQMLVRCAAIIGLT
hsAC	770	KYSIKLTEKLNMTLHSDKESEEVCHLTSGVRLKNLSPPTSLKEISLIQLDSMRSLHQMLVRCAAIIGLT
rsAC	840	FTTELLFEILPCWNMCKMIKALATLVESNMFDCFRSSKQLQALKQNVTTFEVHYRSLSLKSKEGLAYSE
hsAC	840	FTTELLFEILPCWNMCKMIKITLATLVESNIFYCFRNGKELQKALKQNDPSFEVHYRSLSLKPSGMDHGE
rsAC	910	EEQLREMEGEVIECRILRFORPI MQKTAYELWLKDQKVLHLKCARFLEESAHRONHCRNRDFIPYHHFI
hsAC	910	EEQLRELENEVIECHRIIRFONPMQKTAYELWLKDQKAMHLKCARFLEEDAHRDCHCRGRDFIPYHHFI
rsAC	980	ADIRLNTLDMDITVKKMVKSHGFKTEDEVIFSKSEIPR-KFKFPENISITETREKILHFFDMVITKMRISQ
hsAC	980	VNIRLNALDMDAIIKKMAMSHGFKTEKLILSNSEIPETSAFFPENRSPETREKILNFFDMVITKMRISQ
sAC	1049	DDVIPLESCHEELLQIVILPLAQHFVALEENKALYYFLEIASAYLILGDNYNAYMYLGEGERLLKSLT
hsAC	1050	EDIIPLESCQCEEILEIVILPLAHFALGENDKALYYFLEIASAYLIFCDNYMAYMYLNEGOKLLKTLK
sAC	1119	NEOSWSQTFEYATFYSLKGEICFNMQQIVLAKKMLRKALKLLNRMFPONLLSLTFQMHIEKNRSLSHFMNQ
hsAC	1120	KDKSWSQTFESATFYSLKGEICFNMQQIVLAKKMLRKALKLLNRIFFPNLLISLFLHIEKNRSHFHYVNR

3/10

Alignment of human and rat sAC amino acid sequences

FIG. 2B

rsAC 1189	HTQEGSLPGKKLAQLFLQSSCFSLWKIYSLNFFHYKYYGRLAAIMQMNTSLETQNNFQIIKAFIDFS
hsAC 1190	QAQESPPPQKKRLAQLYRQTVQLSLLWRIYSYSYLFHCKYYAHLAMMMQMNTALETQNCFQIIKAYLDYS
rsAC 1258	LYRHLAGYEGVWFKEYEILVMEQLNLPLKGEAFEIMAYAADALGHIFLTGHLDLAIELGSRAHKMWSLL
hsAC 1260	LYHHLAGYKGVWFKEYEVMAMEHIFNLPLKGEIEIVAYVAETLVFNKLIMGHLDLAIELGSRALOMWALL
rsAC 1328	RNPNIYHMYLCLRLSKPLFLKSRYSKHLVQVLGWLWOLSVTEEHIFSKAFFYFVCLDIMLYSGFIYRTFEEC
hsAC 1330	QNPNIHYQSLCLRLSRCLLLNSRYPLIQVLGRLWELSVTQEHIFSKAFFYFVCLDILLYSGFMYRTFEEC
rsAC 1398	LEFIHINEDNRILKFQSGLLLGLYSCLIAMWYARLQEWDFYKFSNRAKTLVTRRTPITVLYYEGISRYMEG
hsAC 1400	LEFIHQYENNRILKFHSGLLLGLYSSVAIWYARLQEWDFYKFSNRAKNLLPRRTMTLTYYDGISRYMEG
rsAC 1468	QVLHLQKQIEEQAENAQDSGVELLKALETLVAQNTTGPVFYPRLYHLMAYVCILMGDGHSCDFLNTALE
hsAC 1470	QVLHLQKQIKEQSENAQASGEELLKNLENLVAQNTTGPVFCPRLYHLMAYVCILMGDGQKCGFLNTALR
rsAC 1538	LSETQGNLLEKCLWSMSKEWWYSAPELTGDQWLQTVLSLPSWDKIVSGNVTILQDVQKNKFLMRVNIIDNP
hsAC 1540	LSETQGNILEKCLWNMKESWYSTSELKEDQWLQTIILSLPSWEKIVAGRVNIQDLQKNKFLMRANTVDNH
rsAC 1608	F
hsAC 1610	F

Human sAC cDNA sequence

4/10

FIG. 3A

ATGAACACTCCAAAAGAAGAATTCCAGGACTGGCCCATAGTCAGAATAGCAGCTCATTTACCAGACCTCATTGTCTATGGACA
TTTCTCCCCAGAGCGACCCCTTTATGGATTATTTTGACGGAGTCCTGATGTTTGTGATATTTACAGGTTTTACTGCAATGACTG
AGAAGTTCAGCAGTGCCATGTACATGGACAGAGGGGCTGAGCAGTTGGTGGAGATCCTCACTACCACATAAGTGAATAGTG
GAGAAAGTGTGATTTTTGGAGGAGACATCCTGAAATTTGCAGGTGATGCACTGCTAGCCCTGTGGAGGGTGGAGCGAAAGCA
GCTGAAAAACATTATCACAGTGGTAATTAATGTAGCCTGGAGATCCATGGATTGTTTGAGACCCAGGAGTGGGAAGAAGGCC
TAGACATCCGAGTCAAGATAGGACTGGCTGCTGGCCACATCAGCATGTTGGTCTTTGGAGATGAAACACACAGCCACTTTCTG
GTGATTGGTCAGGCAGTGGACGATGTGCGCCTTGCCCAAGACATGGCTCAGATGAATGATGTTATTCTGTCAACAACTGCTG
GCAGCTCTGTGACCGGAGCATGATTGAAATTGAGAGTGTTCAGATCAGAGAGCAGTTAAGGTTAACTTCTTAAAAACACCCC
CCAATTTTAATTTTGATGAATTTTACAAAGTGTACGACCTTCATGCATTATTATCCTTCTGGTGAGCACAAAAACCTCCTG
AGGCTTGCAATGCACGCTGAAGCCTGATCCTGAACTGGAGATGTCCCTACAAAAGTATGTGATGGAAGCATTGTTGAAGCAGAT
TGATAACAAACAGCTTCAGGGCTATTTATCTGAGCTTCGCCAGATGACGATTGTGTTGTGAACCTGATGTTGAAGACCAAG
ACAAAGCAGAAGAGATAGGCCAGCCATCCAGGATGCCTATATGCACATCACTTCTGTCTGAAAGATCTTCCAAAGGCCAAATC
AATAAAGTCTTCATGTTTGACAAGGGCTGCTCTTTCTCTGTCTTTGGCTTCCCTGGGGAAAAGGTACCTGACGAGCTCAC
TCATGCTCTGGAATGTGCTATGGATATATTTGACTTCTGCTCTCAAGTCCACAAAATCCAACTGTATCCATCGGTGTTGCCA
GTGGGATTGTCTTCTGTGGGATCGTTGGACACACTGTGAGACACGAGTACACAGTCATTGGTCAAAAAGTCAACTTAGCTGCC
AGGATGATGATGTACTACCCAGGAATTGTGACCTGCGACTCTGCACCTACAATGGGAGCAACCTACCAGCGTACTTTTTTAA
AGAGCTTCCAAAGAAAGTTATGAAAGGTGTTGCAGATTCTGGACCATTGTATCAGTATTGGGGCCGTACTGAGAAAGTCATGT
TTGGTATGGCGTGCCTCATCTGCAACAGAAAGGAGGATTACCTTTGCTGGGACGTAATAAAGAGATCACTACTTCATGTAT
ACTATGAAGAAATTTTGATATCTAACAGCAGCCAAGTCTTAATGTATGAGGGATTACCAGGATATGAAAAAGCCAGATACT
TATGAAAATTGAGTACCTGGCCCAAGGTAAGAATCACAGGATTATTGCCATTTTCATTGAATAAGATCAGCTTCCATCAAACCT
TCTATACCATCCAGATGTTTATGGCCAATGTCCTAGGCCTAGACACTTGTAAACATTATAAAGAACGACAGACCAACCTTCGA
AATAAAGTCATGACACTGTTGGATGAAAAGTTCTACTGTCTTCTTAATGACATTTTCCATGTTTCAGTTCCCTATTTCTCGGGA
GATTTCCAGGATGAGCACCTTGA AAAAGCAAAAACAATTGGAATATTGTTTATGAAGATCTTGAAGCTGATAGTGAAGAGG
AAAGGATTATTTTATCATTGATGAGGCCAGTTTGTGGATTGCACTCCTGGAGATTTATGGAGAAGCTTATCCGGACTCTT
CCTATCTTTCATCATTATGTCCCTGTGTCCCTTCGTTAACATTCCCTGTGCAGCTGCCAGGGCCGTAATAAAGAACAGCAAC
CACCTACATTGTCTGTTGGTGCAGTACAGCCTAACGACATCTCCAAAGATCTGTCTTGACCTCAATGTGAGCTGCATCTCCA
AAGAACTGGACTCGTACCTGGGGAGGGAAGCTGTGGGATTCCATTTTACTGTGAAGAATTGCTTAAAAACCTGGAACATCAT
GAGGTACTCGTTTTCCAACAAACGGAGTCTGAGGAAAAGACAAATAGGACCTGGAATAACCTGTTCAAGTATTCCATTAAGCT
AACAGAGAAGTTAAACATGGTTACTCTCCATAGTGATAAGGAAAGTGAAGAAGTCTGTACCTCACAAGTGGCGTCAGACTGA
AAAACCTGTACCTCCAACGTCATTAAGAAGAAATCTCTCTGATCCAGCTGGATAGCATGAGACTTTCCACCAAATGCTGGTG
AGATGTGCTGCCATCATTGGCCTGACCTTCACCACTGAGTTGTTGTTTGGAGATTCTCCCTGTTGGAATATGAAGATGATGAT
CAAGACCCTGGCAACCCTAGTGAATCTAACATTTTTTATTGTTTCCGGAATGGCAAGGAGCTTCAAAAGGCCCTGAAACAGA
ATGATCCCTCATTTGAGGTGCACTATCGTTCCCTGTCTCTGAAGCCCAAGTGAAGGGATGGATCACGGTGAAGAGGAACAGCTT
CGTGAACCTGGAGAATGAGGTGATCGAGTGGCACAGGATTGATTCTGTAACCCTATGATGCAGAAAACAGCCTACGAGCTGTG
GCTCAAGGACCAGAGAAAAGCCATGCACTTGAATGTGCCCCGCTTTTGAAGAAGATGCCACAGATGTGACCACTGCCGAG
GCAGGGACTTCATTCCTATCATCACTTCACAGTGAATATTCGGCTCAACGCTTTAGACATGGATGCCATTA AAAAGATGGCT
ATGTCTCATGGATTTAAACTGAAGAAAAGCTTATCTTGTTCCAACCTCAGAGATTCCTGAGACATCTGCATTTTTTCTGAAAA
TCGCAGTCTGAAGAAATAAGAGAAAAGATCTTGAATTTCTTTGACCAGTTTTAACAAAAATGAAGACATCTGACGAAGACA
TTATCCCTCTGGAATCTTGCCAGTGTGAAGAAATCCTAGAGATTGTCACTTGCCTCTGGCCCACTTTTCTGGCTTTGGGA
GAAAATGACAAAAGCCTTATATTACTTCTTAGAAATTGCATCTGCTTATCTCATCTTTTGTGATAACTACATGGCATAACATGTA
TTTGAATGAAGGACAGAAGTTGCTAAAAACTCTCAAGAAGGACAAATCTTGAGCCAGACATTTGAGTCTGCCACCTTTTACA
GCCTCAAAGGTGAGGTCTGTTTCAATATGGGCCAGATAGTGCTTGCCAAGAAAATGCTGAGGAAGGCACTGAAGCTCCTCAAC
CGAATCTTTCTTACAACCTTAATCTCCTTGTTTCTCCATATCCATGTGAGAGAAAACAGACACTTTTATTATGTGAATCGGCA
GGCCCAAGAGAGCCACCTCCAGGGAAGAAGAGGCTGGCACAACCTTTACCGGCAAACTGTCTGCCTTTCTTCTGTGGCGCA
TCTATAGCTACAGTTATCTTTTTCACTGCAAGTATTATGCCACCTGGCAGTTATGATGCAAAATGAATACTGCACTGGAACT
CAAAATTGTTTCCAGATCATTAAAGCTTACCTAGACTATTGCTATACCACCACCTGGCTGGCTACAAAGGTGTGTGGTTCAA
ATATGAAGTCATGGCCATGGAGCACATCTTCAACCTCCCCCTGAAAGGCGAGGGCATTGAAATCGTGGCATACGTGGCTGAGA
CACTGGTCTTCAACAAGCTCATAATGGGACACCTGGATTGTTGGCCATTGAGTTAGGCTCCCGAGCCCTTCAGATGTGGGCACTG
CTCCAGAATCCCAACCGACATTATCAGTCCCTCTGCAGACTTAGCAGATGTCTCCTTCTGAACAGCAGATACCCGCAATTGAT
CCAGGTGCTGGGGCGGCTGTGGGAGCTTTCTGTAACACAGGAACACATCTTCAGCAAGGCATTTTTCTATTTTGTCTGCTTGG
ACATCCTGCTTTATTCTGGTTTTGTTTATAGAACATTTGAAGAATGTTTGGAAATTCATACCAATACGAAAAACAAGAAATC
CTCAAGTTCCACAGTGGACTCCTCCTGGGACTTTATTCTCTGTAGCTATCTGGTATGCCAGACTTCAGGAATGGGACAACCTT
TTACAAATTTTCCAATAGAGCTAAAAATCTTTGCCAAGAAGAACCATGACACTTACTTACTATGACGGAATATCTAGGTACA
TGGAGGGGCAAGTTCTTACCTTCAAAAAACAATCAAGAAGACGTGAGAGAATGCCAAGCCAGTGGGGAGGAGCTACTCAAG
AACTTGAGAATCTGGTGGCTCAAAATACCACTGGCCCTGTCTTTGCCAAGGCTCTACC

5/10

HUMAN SAC CDNA SEQUENCE

FIG. 3B

ACCTGATGGCTTACGTCTGTATATTAATGGGAGATGGGCAGAAATGTGGCCTCTTCCTGAACACAGCCT
TGCGGCTCTCTGAAACACAGGGGAATATACTGGAGAAATGCTGGCTGAACATGAACAAAGAATCATGGT
ACTCAACCTCTGAGTTAAAAGAAGACCAATGGCTTCAGACGATCTTGAGTCTCCCATCATGGGAAAAAA
TTGTAGCAGGCAGGGTAAACATTGAGGATCTTCAAAAAAACAATTCCTGATGAGAGCTAATACCGTGG
ACAATCATTTCTAA

6/10

Alignment of the rat and human sAC nucleotide sequences

FIG. 4A

hsAC	1	ATGAACACTCCAAAAGAAGATTCCAGGACTGGCCCATAGTCAGAAATAGCAGCTCACTTACCAGACCTCA
rsAC	1	ATGAGTGCCTGAAGGCAGGAATTACAGGACAGGGCAATCGTCAAGATAGCTGCTCACTTACCGGACCTCA
hsAC	71	TTGTCTATGGACATTTCTCCCCAGAGCGACCCCTTTATGGATTATTTTGACGGAGTCTGATGTTTGTGGA
rsAC	71	TTGTCTATGGACATTTCTCTCCGAGCGGCCGTCAGTGAAATGTTTTGATGGAGTCTGATGTTTGTGGA
hsAC	141	TATTTCAGGTTTTACTGCAATGACTGAGAAGTTCAGCAGTCCCATGTACATGGACAGAGGGGCTGAGCAG
rsAC	141	TATTTCAGGCTTTACTGCAATGACTGAGAAGTTCAGCACAGCCATGTACATGGACCGAGGAGCCGAGCAG
hsAC	211	TTGGTGGAGATCCTCAACTACCACATAAGTGCAATAGTGGAGAAAGTGTGATTTTTGGAGGAGACATCC
rsAC	211	CTGGTGGAGATCCTCAACTACTACATAAGTGCCATAGTGGAGAAAGTACTGATTTTTGGAGGAGACATCC
hsAC	281	TGAAATTTGCAGGTGATGCACTGCTAGCCCTGTGGAGGGTGGAGCGAAAGCAGCTGAAACAACTTATCAC
rsAC	281	TAAATTTGCAGGTGACGCCCTGTGGCCCTGTGGAAAGTGGAAACGAAAGCACTGAAGAAATATCATCAC
hsAC	351	AGTGGTAATTAATGTAGCCTGGAGATCCATGGATTGTTTGAGACCCAGGAGTGGGAAGAAGGCCTAGAC
rsAC	351	GGTGGTAATTAATGTCAGCCTGGAGATTATGGCTTGTGTTGAAGCCAAGGAGGTTGAAGAAGGCCTGGAT
hsAC	421	ATCCGAGTCAAGATAGGACTGGCTGCTGGCCACATCAGCATGTTGGTCTTTGGAGATGAAACACACAGCC
rsAC	421	ATTCGAGTTAAGATAGGACTGGCTGCTGGCCACATCACCATGTTGGTCTTTGGGGATGAAACACGGAACT
hsAC	491	ACTTCTGGTGATTGGTCAGGCAGTGGACGATGTGCGCCTTGCCAGAACATGGCTCAGATGAATGATGT
rsAC	491	ACTTCTGGTGATTGGCCAAAGCGGTGGATGATGTACGCCTTGCTCAGAACATGGCTCAGATGAATGATGT
hsAC	561	TATTCTGTACCAAACCTGCTGGCAGCTCTGTGACCGGAGCATGATTGAAATTCAGAGTGTTCAGATCAG
rsAC	561	TATTCTGTACCAAACCTGCTGGCAGCTCTGTGATCGGAGCATGATTGAAATTCAGAGGATTCCGGATCAG
hsAC	631	AGAGCAGTTAAGGTTAACTTCTTAAACACCCCCCAATTTTAATTTTGATGAATTTTTTACAAAGTGTA
rsAC	631	AGAGCAGTTAAGGTTAGCTTCTTAAACACCCCCCACTTTTAATTTTCGACGAGTTTTTTGCCAAGTGTA
hsAC	701	CGACCTTCATGCATTATTATCCTTCTGGTGACACAAAAACCTCCTGAGGCTTGCTGCAAGCTGAAGCC
rsAC	701	TGGCCTTCATGGATTATTATCCTTCTGGTGACACAAAAACTTCCTAAGGCTTGCTGCAATGCTGGAGTC
hsAC	771	TGATCCTGAACCTGGAGATGTCTCTACAAAAGTATGTGATGGAAGCATTTTGAAGCAGATTGATACAAA
rsAC	771	TGATCCTGAACCTCGAGTTGTCTCTACAAAAGTATGTGATGGAATCATTTTGAAGCAGATTGATGACAAG
hsAC	841	CAGCTTCAGGGCTATTTATCTGAGCTTCGCCAGTGACGATTGTGTTTGTGAACCTGATGTTTGAAGACC
rsAC	841	CAGCTTCAGGGCTATTTATCTGAGCTTCGTCCTGTGACGATCGTGTTTGTGAACCTGATGTTTGAAGACC
hsAC	911	AAGACAAAGCAGAAGAGATAGGCCAGCCATCCAGGATGCCTATATGCACATCACTTCTGTCTGAAGAT
rsAC	911	AAGACAAAGCAGAAGTCATAGGATCAGCCATCCAAAGCTGCCTGTGTGCACATCACTTCCGTCTGAAGGT
hsAC	981	CTTCCAGAGCCAAATCAATAAAGTCTTCATGTTTGACAAAGGCTGCTCTTTCCTCTGTGCTTTGGCTTC
rsAC	981	CTTCCAGAGCCAGATCAATAAAGTCTTCATGTTTGATAAAGGCTGCTCTTTCCTCTGTGCTTTGGTTTC
hsAC	1051	CCTGGGGAAAAGGTACCTGACGAGCTCACTCATGCTCTGGAATGTGCTATGGATATATTTGACTTCTGCT
rsAC	1051	CCTGGGGAAAAGGCCCTGACGAGATCACTCACGCTTTGGAAGTGGCTGGATATATTTGACTTCTGCT
hsAC	1121	CTCAAGTCCACAAAATCCAAACTGTATCCATCGGTGTTGCCAGTGGGATTGTCTTCTGTGGGATCGTTGG
rsAC	1121	CTCAGGTCCACAAAATCCGTACTGTCTCCATCGGCGTCCAGTGGGATTGTCTTCTGTGGGATCGTTGG

7/10

Alignment of the rat and human sAC nucleotide sequences

FIG. 4B

hsAC 1191	ACACACTGTGAGACACGAGTACACAGTCATTGGTCAAAAAGTCAACTT	AGCTGCCAGGATGATGATGTAC
rsAC 1191	ACACACTGTGAGACACGAGTACACAGTCATTGGCCAAAAGTCAATA	TGCTGCCAGGATGATGATGTAT
hsAC 1261	TACCCAGGAATTGTGACCTGCGACTCTGTCAGCTACAATGGGAGCAACCTACCAGCGTACTTTTTTAAAG	
rsAC 1261	TACCCAGGCATCGTGACCTGCGACTCTGTCAGATACGATGGCAGCAACCTGCCAGCGTACTTTTTTAAAG	
hsAC 1331	AGCTTCCAAAGAAAGTTATGAAAGGTGTTGCAGATTCTGGACCAATTGTATCAGTATTGGGGCGTACTGA	
rsAC 1331	AGCTTCCAAAGAAAGTCATGAAAGGAGTTGGCGATCCCGGACCAGTGTATCAGTGTCTGGGCGTCAATGA	
hsAC 1401	GAAAGTCATGTTTGGTATGGCGTGCCTCATCTGCAACAGAAAGGAGGATTACCCTTTTCTGGGACGTAAT	
rsAC 1401	GAAAGTCATGTTTGGTATGGCGTATCTCATCTGCAACAGATATGAGGGCTACCCTTTTCTGGGTCGTGTT	
hsAC 1471	AAAGAGATCAACTACTTTCATGTATACTATGAAGAAATTTTGTATATCTAACAGCAGCCAAAGTCTTAATGT	
rsAC 1471	AGGAGATCGACTATTTTCATGTCTACTATGAAGGACTTTTCTGATGACGAAGTCAGCCGAGTCTTAATGT	
hsAC 1541	ATGAGGGATTACCAGGATATGGAAAAAGCCAGATACTTATGAAAAATTGAGTACCTGGCC---CAAGGTAA	
rsAC 1541	ATGAAGGATTGCCAGGATATGGGAAAAAGCCAGGTACTTATGAAAAATCGAGTATCTGGCCTCCAGCATGA	
hsAC 1608	GAATCAGCAGGATTTATTGCCATTTTCATTGAAATAAGATCAGCTTCCATCAAACTTTCTATACCATCCAGATG	
rsAC 1611	GAAACATAGGGCTGTGCTTATTGCACTGACTAAGATCAGCTTCCATCAAAATTTTACACTATCCAGATA	
hsAC 1678	TTTCATGGCCAAATGTCTAGGCCTAGACACTTGTAAACATTATAAAGAACGACAGACCAACCTTCGAAATA	
rsAC 1681	CTCATGGCTAAAGTACTAGGCTCTGGATACTTGTAAACATTACAAAGAACGACAGACCAATCTTCGAAATA	
hsAC 1748	AAGTCATGACACTGTTGGATGAAAGTTTCTACTGTCTTCTTAATGACATTTTCCATGTTTCAGTTCCTTAT	
rsAC 1751	GAGTCAAGACGCTGTTGGATGATAAATACCACTGTCTCCTTAACGACATCTTCCATGTTTCAGTTCCTCGT	
hsAC 1818	TTCTCGGGAGATTTCAGGATGAGCACCTTGAAAAAGCAAAAACAATTGGAATATTTGTTTATGAAGATC	
rsAC 1821	TTCCCGGGAGATGTCCAGGATGAGCAAGATAAGAAAGCAGAAGCAACTGGAAGCTGTGTTTATGAAGATC	
hsAC 1888	TTGAAGCTGATAGTGAAAGAGGAAAGGATTATTTTATCATTTGATGAGGCCAGTTTGTGGATTGACCT	
rsAC 1891	CTGGAGCAAAACAGTGAGGCAAGAAAGGATTATCTTCATCATGACGAGGCCAGTTTGTGGACGTAGCCT	
hsAC 1958	CCTGGAGATTATATGGAGAAGCTTATCCGGACTCTTCATCTTCATCATTATGTCCCTGTGTCCCTTGGT	
rsAC 1961	CCTGGGCGTTTCATAGAAAAGCTCATCCGGTCCATGCCCATCTTCATTGTTATGTCCCTGTGTCCCTTCCC	
hsAC 2028	TAAACATTCCCTGTGCAGCTGCCAGGGCCGTAATAAAGAACAAGGAACACCACTACATTGTGCTTGGTGCA	
rsAC 2031	TGAAACATCCCTGGCAGCTGCCAATGCCATAATGAAGAACCGGAACACCACTACATCACTCGGTACC	
hsAC 2098	GTACAGCCTAACGACATCTCCAACAAGATCTGTCTTGACCTCAATGTGAGCTGCATCTCCAAGAAGCTGG	
rsAC 2101	ATGCAGCCTCAGGAAATCCGGGACAAGGTCTGTCTTGACCTGAGTGTAAAGCAGCATCCCCAGAGAGCTTG	
hsAC 2168	ACTCGTACCTGGGGAGGGAAAGCTGTGGGATTCCATTTTACTGTGAAGAATTGCTTAAAAACCTGGAAACA	
rsAC 2171	ACTCGTACCTGGTGGAGGGGAGCTGCGGGATTCCGTATTACTGTGAGGAATGCTGAAAAACCTCGACCA	
hsAC 2238	TCATGAGGTACTCGTTTCCAACAACCGAGTCTGAGGAAAAGACAAATAGGACCTGGAATAACCTGTTC	
rsAC 2241	CCACAGAATTCTCATTTTCCAACAAGCAGAGGCTGAGGAAAAGACAAACCTGACCTGGAATAACCTGTTC	

8/10

Alignment of the rat and human sAC nucleotide sequences

FIG. 4C

hsAC 2308	AAGTATTCATTAAAGCTAACAGAGAAGTTAAACATGGTTACTCTCCATAGTGATAAGGAAAGTGAAGAAG
rsAC 2311	AAGTACTCTGTAAAGCCGACAGAAGACATGTATCTGTATACTTCC---ATAGCCGCGGGACAGAAAGAAG
hsAC 2378	TCTGTACCTCACAAGTGGCGTCAGACTGAAAAACCTGTCACCTCCAACGTCAITTAAGAAATCTCTCT
rsAC 2378	CCTGTTACCTTACAAGTGGTGTCAAGCTAAAGAACTTGTACCTCCAACATCGCTCAAAGAAATCTCTCT
hsAC 2448	GATCCAGCTGGATAGCATGAGACTTTCCCAACAAATGCTGGTGAGATGTGCTGCCATCATTGGCTGACC
rsAC 2448	GGTTCAACTGGACAGCATGAGCTTTCCCAATCAGATGCTGGTGAGGTGTGCTGCTATCATTGGTCTAACC
hsAC 2518	TTCACCACTGAGTTGTTGTTTGAGATTCTCCCCTGTTGGAATATGAAGATGATGATCAAGACCCCTGGCAA
rsAC 2518	TTCACCACAGAGCTGCTGTTTGAGATTCTCCCCTGCTGGAACATGAAGATGATGATCAAGGCCCTGGCCA
hsAC 2588	CCCTAGTGGAATCTAACATTTTATTGTTTCCGGAATGGCAAGGAGCTTCAAAAGGCCCTGAAACAGAA
rsAC 2588	CCCTAGTGGAATCAAATGTCTTTGATTGCTTTTCGGAGTAGCAAGACCTTCAACTAGCCTTAAAGCAAAA
hsAC 2658	TGATCCCTCATTGAGGTGCACTATCGTTCTTGTCTCTGAAGCCAGTGAAGGGATTGGATCAGCGTGAA
rsAC 2658	CGTGACCACGTTTGAAGTTCAATTATCGTCTTTGTCCCTGAAGTCCAAGGAAGGGTTAGCTTACAGTGAG
hsAC 2728	GAGGAACAGCTTCGTGAACGGGAGATGAGGTGATCGAGTGCCACAGGATTCGATTCTGTAAACCTATGA
rsAC 2728	GAGGAGCAGCTCCGTGAAATGGAAGGAGAGGTGATTGAATGCCGATCCTTCGGTTCTGCAGACCATAA
hsAC 2798	TGCAGAAACAGCCTACGAGCTGTGGCTCAAGGACCAGAGAAAAGCCATGCACTTGAAATGTGCCCGCTT
rsAC 2798	TGCAGAAGACAGCCTACGAACCTGTGGCTCAAGGACCAGAGAAAAGTCTTGCACTTGAAATGCGCCCGCTT
hsAC 2868	TTTAGAAGAGATGCCACAGATGTGACCACTGCCGAGGCAGGGACTTCATTCCCTATCATCACTTCACA
rsAC 2868	TTTGGAGGAGATGCCCATCGGTGCACCACTGCAGAAACAGAGACTTCATTCCCTTACCACCACTTCATA
hsAC 2938	GTGAATATTCCGCTCAACGCTTTAGACATGGATGCCATTAAAAAGATGGCTATGTCTCATGGATTAAAAA
rsAC 2938	GCGGACATTGCACTCAACACTCTGGACATGGATACTGTCAAGAAGATGGTGAAGTCCACGGATTAAAAA
hsAC 3008	CTGAAGAAAGCTTATCTTGTCCAACCTCAGAGATTCCCTGAGACATCTGCATTTTTCTCGAAATCGCAG
rsAC 3008	CTGAAGACGAGTTCATCTTTCTAAATCAGAGATCCCAGGAATTCAAAT---TCCCGAGAACATCAG
hsAC 3078	TCCTGAAGAAATTAAGAGAAAAGATCTTGAATTTCTTTGACCAACGTTTAAACAAAATGAAGACATCTGAC
rsAC 3075	CATCACAGAAACAAGGGAAAAATCTTGCATTTCTTTGACAAATGTTATCATAAAGATGAGGACGTCTCAG
hsAC 3148	GAAGACATTATCCCTCTGGAATCTTGCCAGTGTGAAGAAATCCTAGAGATTGTCATCTTGCTCTGGCCC
rsAC 3145	GATGATGTATCCCTCTAGAATCGTGCCATTGTGAGGAGCTGCTCCAGATTGTCATCTTGCTCTGGCCC
hsAC 3218	ACCATTTTCTGGCTTTGGCAGAAAATGACAAAGCCTTATATTACTTCTTAGAAATTGCATCTGCTTATCT
rsAC 3215	AGCATTTCTAGCCTTGAAGAGAAAACAACAAAGCCTTGTACTACTTCTTAGAAATTGCATCTGCTTATCT
hsAC 3288	CATCTTTTGTGATAACTACATGGCATAACATGATTTTGAATGAAGGACAGAAAGTTGCTAAAACTCTCAAG
rsAC 3285	CATCCTGGGAGACAACCTATAACGCATAACATGATTTTGGCGAAGGGGAAAGCTGTTGAAATCTCTGACA
hsAC 3358	AAGGACAAATCTTGGAGCCAGACATTTGAGTCTGCCACCTTTTACAGCTCAAAGGTGAGGTCTGTTTTCA
rsAC 3355	AATGAAGATTCTTGGAGTCAGACCTTTGAATATGCTACGTTTTATAGTCTCAAAGGTGAGATCTGTTTTA

9/10

FIG. 4D

Alignment of the rat and human sAC nucleotide sequences

hsAC 3428	ATATGGGCAGATAGTGCTTGCCAAGAAAATGCTGAGGAAGGCACTGAAGCTCCTCAACCGAATCTTTCC
rsAC 3425	ATATGGGACAGATGGTGCTGCCAAGAAAATGCTGAGAAAAGCACTGAAGCTTCTCAACAGAATGTTTCC
hsAC 3498	TTTACAACCTTAATCTCCTTGTTTCTCCATATCCATGTGAGAAAAACAGACACTTTTCATTATGTGAATCGG
rsAC 3495	CTGCAATCTACTCTCCTGACTTTTCCAATGCACATTGAGAAAAACAGACTCTCCCACTTCAATGAACCAAG
hsAC 3568	CAGGCCCAAGAGAGCCACCTCCAGGGAAGAAGAGGCTGGCACAACCTTTACCGGCAAACTGTCTGCTTTT
rsAC 3565	CATACCCAGGAGGGCTCGTCCAGGGAAGAAGCTGGCCCAACTTTTCTGCACTGCTCTGCTTCT
hsAC 3638	CCCTTGCTGTGGCGCATCTATAGCTACAGTTATCTTTTCACTGCAAGTATATGCCCACCTGGCAGTTAT
rsAC 3632	CCCTGCTGTGGAAGATCTATAGCTTGAAGTTCTTTTCCACTACAAGTACTATGCTGCTGTCGAGCAAT
hsAC 3708	GATGCAAAATGAATACTGCACTTGAAACTCAAAATTTGTTTCCAGATCATTAAGGCTTACCTAGACTATTTCG
rsAC 3702	AATGCAGATGAACACCTCGTTAGAAACTCAAAACAATTTCCAGATCATCAAGGCTTTCCTGGACTTTTCC
hsAC 3778	CTATACCACCACCTGGCTGGCTACAAAGGTGTGTGGTTCAAATATGAAGTCAATGGCCATGGAGCACATCT
rsAC 3772	CTGTACCGCCATCTGGCTGGATACGAGGGCGTGTGGTTCAAATATGAATCCTGGTCATGGAGCAGCTCT
hsAC 3848	TCAACCTCCCCCTGAAAGGCGAGGGCATTGAAATCGTGGCATACGTGGCTGAGACACTGGTCTTCAACAA
rsAC 3842	TGAACCTCCCCCTGAAAGGCGAAGCCTTTGAAATCATGGCCTATGCAGCCGAGGCACTGGGCCATATCAA
hsAC 3918	GCTCATTAATGGGACACCTGGATTGGCCATTGAGTTAGGCTCCCGAGCCCTTCAGATGTGGGCACTGCTC
rsAC 3912	GTTCTTAACCGGTCACTGGACTTGGCCATTGAATAGGCTCCCGAGCTCACAAGATGTGGTCACTTCTC
hsAC 3988	CAGAATCCCAACCGACATTATCAGTCCCTCTGCAGACTTAGCAGATGTCTCCTTCTGAACAGCAGATACC
rsAC 3982	CGGAATCCCAACAAATACCATATGGTCTCTGCAGACTGAGTAAACCTCTTTTCTTGAAGAGCAGATACA
hsAC 4058	CCCAATTGATCCAGGTGCTGGGCGGGCTGTGGGAGCTTTCTGTAACACAGGAACACATCTTCAGCAAGGC
rsAC 4052	AGCATTTGGTCCAGGTGCTGGGATGGTGTGGGAGCTTTCTGTAACAGAGGAGCACATCTTCAGCAAGGC
hsAC 4128	ATTTTTCTATTTTGTCTGCTTGACATCTGCTTTATTCTGGTTTTTGTATTAGAACATTTGAAGAATGT
rsAC 4122	ATTTTTCTATTTCTGCTGCTTGACATCATGCTTTATTCTGGCTTCAATTACAGAACATTTGAAGAATGT
hsAC 4198	TTGGAATTCATACACCAATACGAAAACAACAGAATCCTCAAGTTCCAAGTGGACTCCTCCTGGGACTTTT
rsAC 4192	TTGGAATTCATACACCACAATGAAGACAACAGAATCCTCAAGTTCCAAGCGGACTCCTCCTGGGACTTTT
hsAC 4268	ATTCTCTCTGTAGCTATCTGGTATGCCAGACTTCAGGAATGGGACAACTTTTACAAATTTTCCAATAGAGC
rsAC 4262	ACTCCTGCAATAGCTGTCTGGTACGCCAGACTTCAGGAATGGGACAACTTTTACAAATTTTCCAATAGAGC
hsAC 4338	TAAAAATCTTTTGGCAAGAAGAACCATGACACTTACTTACTATGACGGAATATCTAGGTACATGGAGGGG
rsAC 4332	GAAGACTTTTAGTGACTGAAGAACCCTAACGGTCTTACTACGAAGGAATTTCTAGGTATATGGAAGGG
hsAC 4408	CAAGTTCTTCACCTTCAAAAACAAATCAAAGAACAGTCAGAGAATGCCAAGCCAGTGGGGAGGAGCTAC
rsAC 4402	CAAGTCTTCATCTTCAGAACAAATAGAAGACAGGCCGAGAATGCTCAGGACAGTGGGGTGGAGCTAC
hsAC 4478	TCAAGAACTTGGAGAACTGCTGGCTCAAAATACCACTGGCCCTGTCTTTTGGCCAAGGCTCTACCACCT
rsAC 4472	TTAAGGCCCTTAGAGACCTTGTGGCTCAAAATACCACTGGCCCGTCTTCTACCCAGGCTCTACCAATTT

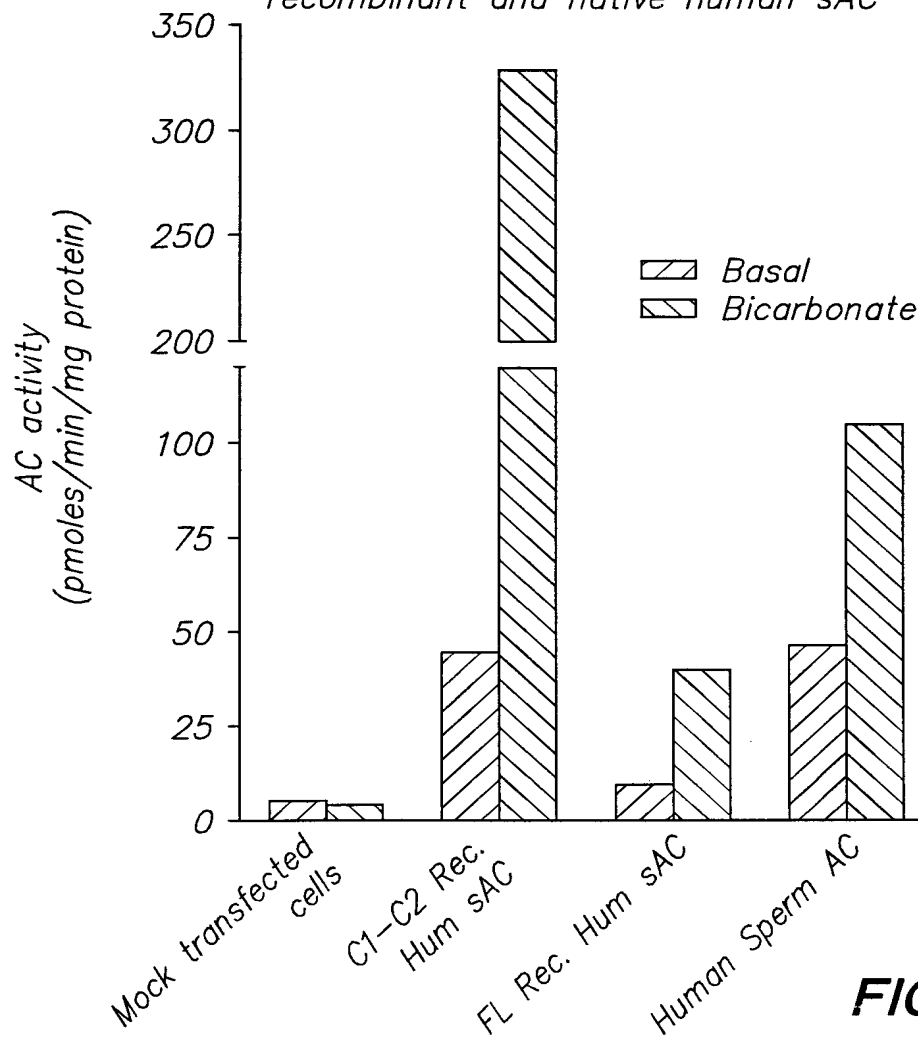
10/10

Alignment of the rat and human sAC nucleotide sequences

FIG. 4E

hsAC 4548	GATGGCTTACGTCTGTATATTAAATGGGAGATGGGCAGAAATGTGGCCTCTTCCTGAACACAGCCTTGCGG
rsAC 4542	GATGGCCTATGTCTGTATACTGATGGGAGACGGGCACAGTTGTGACTTCTTCCTAAACACAGCCTTGAG
hsAC 4618	CTCTCTGAACACAGGGGAATATACTGGAGAAATGCTGGCTGAACATGAACAAAGAATCATGGTACTCAA
rsAC 4612	CTCTCTGAGACACAGGGGAATTTGCTGGAGAAATGTTGGCTGAGCATGAGTAAGGAATGGTGGTACTCAG
hsAC 4688	CCCTCTGAGTTAAAGAAGACCAATGGCTTCAGACGATCTTGAGTCTCCCATCATGGGAATAAAATTGTAGC
rsAC 4682	CCCCCGAGTTGACAGGAGATCAATGGCTTCAGACAGTCTTGAGTCTCCCATCGTGGGATAAAATTGTATC
hsAC 4758	AGGCAGGGTAAACATTCAGGATCTTCAAAAAAACAATTCTTGATGAGAGCTAATACCGTGGACAATCAT
rsAC 4752	AGGCAACGTAACCTTCAGGATGTTCAAAAAAACAATTCTTGATGAGAGTTAATAATCTTGGACAATCCT
hsAC 4828	TTCTAA-----
rsAC 4822	TTCTAATAATTATGAATGAGAACAAGATTGCA

Effect of Bicarbonate (50mM) on the recombinant and native human sAC

**FIG. 5**

SEQUENCE LISTING

<110> Conti, Marco
Jaiswal, Bijay Shankar

<120> Polynucleotides Encoding Human Soluble
Adenylyl Cyclase, Polypeptides Encoded Thereby, and Methods
of Use Thereof

<130> STAN-135WO

<150> 60/155,302

<151> 1999-09-21

<150> 60/191,327

<151> 2000-03-22

<160> 4

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 4833

<212> DNA

<213> Homo sapiens

<400> 1

atgaacactc	caaaagaaga	attccaggac	tggcccatag	tcagaatagc	agctcattta	60
ccagacctca	ttgtctatgg	acatttctcc	ccagagcgac	cctttatgga	ttattttgac	120
ggagtcctga	tgtttgttga	tatttcagggt	tttactgcaa	tgactgagaa	gttcagcagt	180
gccatgtaca	tggacagagg	ggctgagcag	ttggtggaga	tcctcaacta	ccacataagt	240
gcaatagtgg	agaaagtgtt	gattttttgga	ggagacatcc	tgaaatttgc	aggtgatgca	300
ctgctagccc	tgtggagggt	ggagcgaaag	cagctgaaaa	acattatcac	agtggtaatt	360
aaatgtagcc	tggagatcca	tggattgttt	gagacccagg	agtgggaaga	aggcctagac	420
atccgagtc	agataggact	ggctgctggc	cacatcagca	tgttggtctt	tggagatgaa	480
acacacagcc	actttctggt	gatttggtcag	gcagtgagcg	atgtgcgcct	tgcccagaac	540
atggctcaga	tgaatgatgt	tattctgtca	ccaaactgct	ggcagctctg	tgaccggagc	600
atgattgaaa	ttgagagtgt	tccagatcag	agagcagtta	aggtttaact	cttaaaacca	660
cccccaatt	ttaattttga	tgaatttttc	acaaagtgtg	cgaccttcac	gcattattat	720
ccttctggtg	agcacaaaaa	cctcctgagg	cttgcacgca	cgctgaagcc	tgatcctgaa	780
ctggagatgt	ccctacaaaa	gtatgtgatg	gaaagcattt	tgaagcagat	tgataacaaa	840
cagcttcagg	gctattttatc	tgagcttcgc	ccagtgacga	ttgtgtttgt	gaacctgatg	900
tttgaagacc	aagacaaagc	agaagagata	ggcccagcca	tccaggatgc	ctatatgcac	960
atcacttctg	tcctgaagat	cttccaaggc	caaatcaata	aagtcttcac	gtttgacaag	1020
ggctgctctt	tcctctgtgt	ctttggcttc	cctggggaaa	aggtaacctg	cgagctcact	1080
catgctctgg	aatgtgctat	ggatatattt	gacttctgct	ctcaagtcca	caaaatccaa	1140
actgtatcca	tcggtgttgc	cagtgggatt	gtcttctgtg	ggatcgttgg	acacactgtg	1200
agacacgagt	acacagtcac	tgggtcaaaaa	gtcaacttag	ctgccaggat	gatgatgtac	1260
taccacaggaa	ttgtgacctg	cgactctgtc	acctacaatg	ggagcaacct	accagcgta	1320
ttttttaaag	agcttccaaa	gaaagtattg	aaaggtgttg	cagattctgg	accattgtat	1380
cagtattggg	gccgtactga	gaaagtcacg	tttggtatgg	cgtgcctcat	ctgcaacaga	1440
aaggaggatt	accctttgct	gggacgtaat	aaagagatca	actacttcac	gtatactatg	1500
aagaaatttt	tgatatctaa	cagcagccaa	gtcttaatgt	atgagggatt	accaggatat	1560
ggaaaaagcc	agatacttat	gaaaattgag	tacctggccc	aaggtaagaa	tcacaggatt	1620
attgccattt	cattgaataa	gatcagcttc	catcaaaact	tctataccat	ccagatgttc	1680
atggccaatg	tcctaggcct	agacacttgt	aaacattata	aagaacgaca	gaccaacctt	1740
cgaaataaag	tcatgacact	gttggatgaa	aagttctact	gtcttcttaa	tgacattttc	1800
catgttcagt	tcctattttc	tcgggagatt	tccaggatga	gcaccttgaa	aaagcaaaaa	1860
caattggaaa	tattgtttat	gaagatcttg	aagctgatag	tgaaagagga	aaggattatt	1920
tttatcattg	atgaggccca	gtttgtggat	tcgacctcct	ggagatttat	ggagaagctt	1980
atccggactc	ttcctatctt	catcattatg	tcctgtgtgc	ccttcgttaa	cattccctgt	2040
gcagctgcc	gggcccgaat	aaagaacagg	aacaccacct	acattgtcgt	tggtgcagta	2100
cagcctaacg	acatctccaa	caagatctgt	cttgacctca	atgtgagctg	catctccaaa	2160

gaactggact	cgtacctggg	ggaggggaagc	tgtggggattc	catttttactg	tgaagaattg	2220
cttaaaaaacc	tggaacatca	tgaggtactc	gtttttccaac	aaacggagtc	tgaggaaaag	2280
acaaatagga	cctggaataa	cctgttcaag	tattccatta	agctaacaga	gaagttaaacc	2340
atggttactc	tccatagtga	taaggaaagt	gaagaagtct	gtcacctcac	aagtggcgtc	2400
agactgaaaa	acctgtcacc	tccaacgtca	ttaaaaagaa	tctctctgat	ccagctggat	2460
agcatgagac	tttcccacca	aatgctgggtg	agatgtgctg	ccatcattgg	cctgaccttc	2520
accactgagt	tgttggttga	gattctcccc	tgttggaata	tgaagatgat	gatcaagacc	2580
ctggcaacc	tagtggaaatc	taacattttt	tattgtttcc	ggaatggcaa	ggagcttcaa	2640
aaggccctga	aacagaatga	tccctcattt	gaggtgcact	atcgttcctt	gtctctgaag	2700
cccagtgaag	ggatggatca	cggatgaagag	gaacagcttc	gtgaactgga	gaatgaggtg	2760
atcgagtgcc	acaggattcg	attctgtaac	cctatgatgc	agaaaacagc	ctacgagctg	2820
tggctcaagg	accagagaaa	agccatgcac	ttgaaatgtg	cccgtttttt	agaagaagat	2880
gcccacagat	gtgacctctg	ccgaggcagg	gacttcattc	cctatcatca	cttcacagtg	2940
aatatctcggc	tcaacgcttt	agacatggat	gccattaaaa	agatggctat	gtctcatgga	3000
tttaaaactg	aagaaaagct	tatcttgtcc	aactcagaga	ttcctgagac	atctgcattc	3060
tttctgaaa	atcgcagtc	tgaagaaata	agagaaaaga	tcttgaattt	ctttgaccac	3120
gttttaacaa	aaatgaagac	atctgacgaa	gacattatcc	ctctggaatc	ttgccagtgt	3180
gaagaaatcc	tagagattgt	catcttgcct	ctggcccacc	atcttctggc	tttgggagaa	3240
aatgacaaag	cottatatta	cttcttagaa	attgcatctg	cttatctcat	cttttgtgat	3300
aactacatgg	catacatgta	tttgaatgaa	ggacagaagt	tgctaaaaaac	tctcaagaag	3360
gacaaatctt	ggagccagac	atttgagctc	gccacctttt	acagcctcaa	aggtgaggtc	3420
tgtttcaata	tgggcccagat	agtgcctgtcc	aagaaaatgc	tgagggaaggc	actgaagctc	3480
ctcaaccgaa	tctttcctta	caacttaatc	tcttgttttc	tccatatcca	tgctcgagaaa	3540
aacagacact	ttcattatgt	gaatcggcag	gcccagagaa	gcccacctcc	aggggaagaag	3600
aggctggcac	aactttaccg	gcaaaactgtc	tgcttttctc	tgctgtggcg	catctatagc	3660
tacagttatc	tttttctactg	caagtattat	gcccacctgg	cagttatgat	gcaaatgaat	3720
actgcactgg	aaactcaaaa	ttgtttccag	atcattaagg	cttacctaga	ctattcgcta	3780
taccaccacc	tggctggcta	caaagggtgtg	tggttcaa	atgaagtc	ggccatggag	3840
cacatcttca	acctccccct	gaaaggcgag	ggcattgaaa	tcgtggcata	cgctggctgag	3900
acactgggtct	tcaacaagct	cataatggga	cacctggatt	tggccattga	gttaggctcc	3960
cgagcccttc	agatgtgggc	actgctccag	aatcccaacc	gacattatca	gtccctctgc	4020
agacttagca	gatgtctcct	tctgaacagc	agatacccg	aattgatcca	ggtgctgggg	4080
cggctgtggg	agctttctgt	aacacaggaa	cacatcttca	gcaaggcatt	tttctatttt	4140
gtctgcttgg	acatcctgct	ttattctggt	tttgtttata	gaacatttga	agaatgtttg	4200
gaattcatac	accaatacga	aaacaacaga	atcctcaagt	tccacagtgg	actcctcctg	4260
ggacttttatt	cctctgtagc	tatctggtat	gccagacttc	aggaatggga	caacttttac	4320
aaattttcca	atagagctaa	aaatcttttg	ccaagaagaa	ccatgacact	tacttactat	4380
gacggaatat	ctaggtacat	ggagggggcaa	gttcttcacc	ttcaaaaaaca	aatcaaaagaa	4440
cagtcaagaa	atgcccgaagc	cagtgggggag	gagctactca	agaacttgga	gaatctggtg	4500
gctcaaaaata	ccactggccc	tgtcttttgc	ccaaggctct	accacctgat	ggcttacgctc	4560
tgtatatata	tgggagatgg	gcagaaatgt	ggcctcttcc	tgaacacagc	cttgccgctc	4620
tctgaaacac	aggggaatat	actggagaaa	tgctggctga	acatgaacaa	agaatcatgg	4680
tactcaacct	ctgagttaaa	agaagaccaa	tggcttcaga	cgatcttgag	tctcccatca	4740
tgggaaaaaaa	ttgtagcagg	cagggtaaac	attcaggatc	ttcaaaaaaaa	caaattcctg	4800
atgagagcta	ataccgtgga	caatcatttc	taa			4833

<210> 2

<211> 1610

<212> PRT

<213> Homo Sapiens

<400> 2

Met	Asn	Thr	Pro	Lys	Glu	Glu	Phe	Gln	Asp	Trp	Pro	Ile	Val	Arg	Ile
1				5				10					15		
Ala	Ala	His	Leu	Pro	Asp	Leu	Ile	Val	Tyr	Gly	His	Phe	Ser	Pro	Glu
			20					25					30		
Arg	Pro	Phe	Met	Asp	Tyr	Phe	Asp	Gly	Val	Leu	Met	Phe	Val	Asp	Ile
		35					40					45			
Ser	Gly	Phe	Thr	Ala	Met	Thr	Glu	Lys	Phe	Ser	Ser	Ala	Met	Tyr	Met
	50					55					60				
Asp	Arg	Gly	Ala	Glu	Gln	Leu	Val	Glu	Ile	Leu	Asn	Tyr	His	Ile	Ser
65					70					75				80	

Ala	Ile	Val	Glu	Lys	Val	Leu	Ile	Phe	Gly	Gly	Asp	Ile	Leu	Lys	Phe
				85					90					95	
Ala	Gly	Asp	Ala	Leu	Leu	Ala	Leu	Trp	Arg	Val	Glu	Arg	Lys	Gln	Leu
			100					105					110		
Lys	Asn	Ile	Ile	Thr	Val	Val	Ile	Lys	Cys	Ser	Leu	Glu	Ile	His	Gly
		115					120					125			
Leu	Phe	Glu	Thr	Gln	Glu	Trp	Glu	Glu	Gly	Leu	Asp	Ile	Arg	Val	Lys
	130					135					140				
Ile	Gly	Leu	Ala	Ala	Gly	His	Ile	Ser	Met	Leu	Val	Phe	Gly	Asp	Glu
	145				150					155					160
Thr	His	Ser	His	Phe	Leu	Val	Ile	Gly	Gln	Ala	Val	Asp	Asp	Val	Arg
				165					170					175	
Leu	Ala	Gln	Asn	Met	Ala	Gln	Met	Asn	Asp	Val	Ile	Leu	Ser	Pro	Asn
			180					185					190		
Cys	Trp	Gln	Leu	Cys	Asp	Arg	Ser	Met	Ile	Glu	Ile	Glu	Ser	Val	Pro
		195					200					205			
Asp	Gln	Arg	Ala	Val	Lys	Val	Asn	Phe	Leu	Lys	Pro	Pro	Pro	Asn	Phe
	210					215					220				
Asn	Phe	Asp	Glu	Phe	Phe	Thr	Lys	Cys	Thr	Thr	Phe	Met	His	Tyr	Tyr
	225				230					235					240
Pro	Ser	Gly	Glu	His	Lys	Asn	Leu	Leu	Arg	Leu	Ala	Cys	Thr	Leu	Lys
				245					250					255	
Pro	Asp	Pro	Glu	Leu	Glu	Met	Ser	Leu	Gln	Lys	Tyr	Val	Met	Glu	Ser
			260					265					270		
Ile	Leu	Lys	Gln	Ile	Asp	Asn	Lys	Gln	Leu	Gln	Gly	Tyr	Leu	Ser	Glu
		275					280								
Leu	Arg	Pro	Val	Thr	Ile	Val	Phe	Val	Asn	Leu	Met	Phe	Glu	Asp	Gln
	290					295					300				
Asp	Lys	Ala	Glu	Glu	Ile	Gly	Pro	Ala	Ile	Gln	Asp	Ala	Tyr	Met	His
	305				310					315					320
Ile	Thr	Ser	Val	Leu	Lys	Ile	Phe	Gln	Gly	Gln	Ile	Asn	Lys	Val	Phe
				325					330					335	
Met	Phe	Asp	Lys	Gly	Cys	Ser	Phe	Leu	Cys	Val	Phe	Gly	Phe	Pro	Gly
			340					345					350		
Glu	Lys	Val	Pro	Asp	Glu	Leu	Thr	His	Ala	Leu	Glu	Cys	Ala	Met	Asp
		355					360					365			
Ile	Phe	Asp	Phe	Cys	Ser	Gln	Val	His	Lys	Ile	Gln	Thr	Val	Ser	Ile
	370					375					380				
Gly	Val	Ala	Ser	Gly	Ile	Val	Phe	Cys	Gly	Ile	Val	Gly	His	Thr	Val
	385				390					395					400
Arg	His	Glu	Tyr	Thr	Val	Ile	Gly	Gln	Lys	Val	Asn	Leu	Ala	Ala	Arg
				405					410					415	
Met	Met	Met	Tyr	Tyr	Pro	Gly	Ile	Val	Thr	Cys	Asp	Ser	Val	Thr	Tyr
			420					425					430		
Asn	Gly	Ser	Asn	Leu	Pro	Ala	Tyr	Phe	Phe	Lys	Glu	Leu	Pro	Lys	Lys
		435					440					445			
Val	Met	Lys	Gly	Val	Ala	Asp	Ser	Gly	Pro	Leu	Tyr	Gln	Tyr	Trp	Gly
	450					455					460				
Arg	Thr	Glu	Lys	Val	Met	Phe	Gly	Met	Ala	Cys	Leu	Ile	Cys	Asn	Arg
	465				470					475					480
Lys	Glu	Asp	Tyr	Pro	Leu	Leu	Gly	Arg	Asn	Lys	Glu	Ile	Asn	Tyr	Phe
				485					490					495	
Met	Tyr	Thr	Met	Lys	Lys	Phe	Leu	Ile	Ser	Asn	Ser	Ser	Gln	Val	Leu
			500					505					510		
Met	Tyr	Glu	Gly	Leu	Pro	Gly	Tyr	Gly	Lys	Ser	Gln	Ile	Leu	Met	Lys
		515					520					525			
Ile	Glu	Tyr	Leu	Ala	Gln	Gly	Lys	Asn	His	Arg	Ile	Ile	Ala	Ile	Ser
	530					535					540				
Leu	Asn	Lys	Ile	Ser	Phe	His	Gln	Thr	Phe	Tyr	Thr	Ile	Gln	Met	Phe
	545				550					555					560
Met	Ala	Asn	Val	Leu	Gly	Leu	Asp	Thr	Cys	Lys	His	Tyr	Lys	Glu	Arg
				565					570					575	

Gln	Thr	Asn	Leu	Arg	Asn	Lys	Val	Met	Thr	Leu	Leu	Asp	Glu	Lys	Phe		
			580					585					590				
Tyr	Cys	Leu	Leu	Asn	Asp	Ile	Phe	His	Val	Gln	Phe	Pro	Ile	Ser	Arg		
		595					600					605					
Glu	Ile	Ser	Arg	Met	Ser	Thr	Leu	Lys	Lys	Gln	Lys	Gln	Leu	Glu	Ile		
	610					615					620						
Leu	Phe	Met	Lys	Ile	Leu	Lys	Leu	Ile	Val	Lys	Glu	Glu	Arg	Ile	Ile		
625					630					635					640		
Phe	Ile	Ile	Asp	Glu	Ala	Gln	Phe	Val	Asp	Ser	Thr	Ser	Trp	Arg	Phe		
			645						650					655			
Met	Glu	Lys	Leu	Ile	Arg	Thr	Leu	Pro	Ile	Phe	Ile	Ile	Met	Ser	Leu		
			660					665					670				
Cys	Pro	Phe	Val	Asn	Ile	Pro	Cys	Ala	Ala	Ala	Arg	Ala	Val	Ile	Lys		
		675					680					685					
Asn	Arg	Asn	Thr	Thr	Tyr	Ile	Val	Val	Gly	Ala	Val	Gln	Pro	Asn	Asp		
	690				695						700						
Ile	Ser	Asn	Lys	Ile	Cys	Leu	Asp	Leu	Asn	Val	Ser	Cys	Ile	Ser	Lys		
705					710					715					720		
Glu	Leu	Asp	Ser	Tyr	Leu	Gly	Glu	Gly	Ser	Cys	Gly	Ile	Pro	Phe	Tyr		
			725					730						735			
Cys	Glu	Glu	Leu	Leu	Lys	Asn	Leu	Glu	His	His	Glu	Val	Leu	Val	Phe		
			740					745					750				
Gln	Gln	Thr	Glu	Ser	Glu	Glu	Lys	Thr	Asn	Arg	Thr	Trp	Asn	Asn	Leu		
		755					760					765					
Phe	Lys	Tyr	Ser	Ile	Lys	Leu	Thr	Glu	Lys	Leu	Asn	Met	Val	Thr	Leu		
	770				775					780							
His	Ser	Asp	Lys	Glu	Ser	Glu	Glu	Val	Cys	His	Leu	Thr	Ser	Gly	Val		
785					790				795						800		
Arg	Leu	Lys	Asn	Leu	Ser	Pro	Pro	Thr	Ser	Leu	Lys	Glu	Ile	Ser	Leu		
			805					810						815			
Ile	Gln	Leu	Asp	Ser	Met	Arg	Leu	Ser	His	Gln	Met	Leu	Val	Arg	Cys		
			820					825					830				
Ala	Ala	Ile	Ile	Gly	Leu	Thr	Phe	Thr	Thr	Glu	Leu	Leu	Phe	Glu	Ile		
		835					840					845					
Leu	Pro	Cys	Trp	Asn	Met	Lys	Met	Met	Ile	Lys	Thr	Leu	Ala	Thr	Leu		
	850					855				860							
Val	Glu	Ser	Asn	Ile	Phe	Tyr	Cys	Phe	Arg	Asn	Gly	Lys	Glu	Leu	Gln		
865					870					875					880		
Lys	Ala	Leu	Lys	Gln	Asn	Asp	Pro	Ser	Phe	Glu	Val	His	Tyr	Arg	Ser		
			885						890					895			
Leu	Ser	Leu	Lys	Pro	Ser	Glu	Gly	Met	Asp	His	Gly	Glu	Glu	Glu	Gln		
			900					905					910				
Leu	Arg	Glu	Leu	Glu	Asn	Glu	Val	Ile	Glu	Cys	His	Arg	Ile	Arg	Phe		
		915					920					925					
Cys	Asn	Pro	Met	Met	Gln	Lys	Thr	Ala	Tyr	Glu	Leu	Trp	Leu	Lys	Asp		
	930					935					940						
Gln	Arg	Lys	Ala	Met	His	Leu	Lys	Cys	Ala	Arg	Phe	Leu	Glu	Glu	Asp		
945					950					955					960		
Ala	His	Arg	Cys	Asp	His	Cys	Arg	Gly	Arg	Asp	Phe	Ile	Pro	Tyr	His		
			965						970					975			
His	Phe	Thr	Val	Asn	Ile	Arg	Leu	Asn	Ala	Leu	Asp	Met	Asp	Ala	Ile		
			980					985					990				
Lys	Lys	Met	Ala	Met	Ser	His	Gly	Phe	Lys	Thr	Glu	Glu	Lys	Leu	Ile		
		995					1000					1005					
Leu	Ser	Asn	Ser	Glu	Ile	Pro	Glu	Thr	Ser	Ala	Phe	Phe	Pro	Glu	Asn		
	1010					1015					1020						
Arg	Ser	Pro	Glu	Glu	Ile	Arg	Glu	Lys	Ile	Leu	Asn	Phe	Phe	Asp	His		
1025					1030					1035					1040		
Val	Leu	Thr	Lys	Met	Lys	Thr	Ser	Asp	Glu	Asp	Ile	Ile	Pro	Leu	Glu		
			1045						1050					1055			
Ser	Cys	Gln	Cys	Glu	Glu	Ile	Leu	Glu	Ile	Val	Ile	Leu	Pro	Leu	Ala		
			1060					1065					1070				

His	His	Phe	Leu	Ala	Leu	Gly	Glu	Asn	Asp	Lys	Ala	Leu	Tyr	Tyr	Phe		
		1075					1080					1085					
Leu	Glu	Ile	Ala	Ser	Ala	Tyr	Leu	Ile	Phe	Cys	Asp	Asn	Tyr	Met	Ala		
		1090					1095					1100					
Tyr	Met	Tyr	Leu	Asn	Glu	Gly	Gln	Lys	Leu	Leu	Lys	Thr	Leu	Lys	Lys		
1105					1110						1115				1120		
Asp	Lys	Ser	Trp	Ser	Gln	Thr	Phe	Glu	Ser	Ala	Thr	Phe	Tyr	Ser	Leu		
				1125						1130					1135		
Lys	Gly	Glu	Val	Cys	Phe	Asn	Met	Gly	Gln	Ile	Val	Leu	Ala	Lys	Lys		
			1140						1145					1150			
Met	Leu	Arg	Lys	Ala	Leu	Lys	Leu	Leu	Asn	Arg	Ile	Phe	Pro	Tyr	Asn		
		1155					1160						1165				
Leu	Ile	Ser	Leu	Phe	Leu	His	Ile	His	Val	Glu	Lys	Asn	Arg	His	Phe		
		1170				1175						1180					
His	Tyr	Val	Asn	Arg	Gln	Ala	Gln	Glu	Ser	Pro	Pro	Pro	Gly	Lys	Lys		
1185					1190						1195				1200		
Arg	Leu	Ala	Gln	Leu	Tyr	Arg	Gln	Thr	Val	Cys	Leu	Ser	Leu	Leu	Trp		
				1205						1210					1215		
Arg	Ile	Tyr	Ser	Tyr	Ser	Tyr	Leu	Phe	His	Cys	Lys	Tyr	Tyr	Ala	His		
			1220					1225						1230			
Leu	Ala	Val	Met	Met	Gln	Met	Asn	Thr	Ala	Leu	Glu	Thr	Gln	Asn	Cys		
		1235					1240						1245				
Phe	Gln	Ile	Ile	Lys	Ala	Tyr	Leu	Asp	Tyr	Ser	Leu	Tyr	His	His	Leu		
	1250				1255							1260					
Ala	Gly	Tyr	Lys	Gly	Val	Trp	Phe	Lys	Tyr	Glu	Val	Met	Ala	Met	Glu		
1265				1270						1275					1280		
His	Ile	Phe	Asn	Leu	Pro	Leu	Lys	Gly	Glu	Gly	Ile	Glu	Ile	Val	Ala		
			1285						1290					1295			
Tyr	Val	Ala	Glu	Thr	Leu	Val	Phe	Asn	Lys	Leu	Ile	Met	Gly	His	Leu		
		1300						1305					1310				
Asp	Leu	Ala	Ile	Glu	Leu	Gly	Ser	Arg	Ala	Leu	Gln	Met	Trp	Ala	Leu		
		1315					1320						1325				
Leu	Gln	Asn	Pro	Asn	Arg	His	Tyr	Gln	Ser	Leu	Cys	Arg	Leu	Ser	Arg		
	1330				1335						1340						
Cys	Leu	Leu	Leu	Asn	Ser	Arg	Tyr	Pro	Gln	Leu	Ile	Gln	Val	Leu	Gly		
1345				1350						1355					1360		
Arg	Leu	Trp	Glu	Leu	Ser	Val	Thr	Gln	Glu	His	Ile	Phe	Ser	Lys	Ala		
			1365						1370					1375			
Phe	Phe	Tyr	Phe	Val	Cys	Leu	Asp	Ile	Leu	Leu	Tyr	Ser	Gly	Phe	Val		
			1380					1385						1390			
Tyr	Arg	Thr	Phe	Glu	Glu	Cys	Leu	Glu	Phe	Ile	His	Gln	Tyr	Glu	Asn		
		1395					1400						1405				
Asn	Arg	Ile	Leu	Lys	Phe	His	Ser	Gly	Leu	Leu	Leu	Gly	Leu	Tyr	Ser		
	1410				1415								1420				
Ser	Val	Ala	Ile	Trp	Tyr	Ala	Arg	Leu	Gln	Glu	Trp	Asp	Asn	Phe	Tyr		
1425				1430						1435					1440		
Lys	Phe	Ser	Asn	Arg	Ala	Lys	Asn	Leu	Leu	Pro	Arg	Arg	Thr	Met	Thr		
			1445						1450					1455			
Leu	Thr	Tyr	Tyr	Asp	Gly	Ile	Ser	Arg	Tyr	Met	Glu	Gly	Gln	Val	Leu		
			1460					1465					1470				
His	Leu	Gln	Lys	Gln	Ile	Lys	Glu	Gln	Ser	Glu	Asn	Ala	Gln	Ala	Ser		
		1475					1480						1485				
Gly	Glu	Glu	Leu	Leu	Lys	Asn	Leu	Glu	Asn	Leu	Val	Ala	Gln	Asn	Thr		
	1490				1495							1500					
Thr	Gly	Pro	Val	Phe	Cys	Pro	Arg	Leu	Tyr	His	Leu	Met	Ala	Tyr	Val		
1505				1510						1515					1520		
Cys	Ile	Leu	Met	Gly	Asp	Gly	Gln	Lys	Cys	Gly	Leu	Phe	Leu	Asn	Thr		
			1525						1530					1535			
Ala	Leu	Arg	Leu	Ser	Glu	Thr	Gln	Gly	Asn	Ile	Leu	Glu	Lys	Cys	Trp		
			1540					1545					1550				
Leu	Asn	Met	Asn	Lys	Glu	Ser	Trp	Tyr	Ser	Thr	Ser	Glu	Leu	Lys	Glu		
		1555					1560						1565				

Asp Gln Trp Leu Gln Thr Ile Leu Ser Leu Pro Ser Trp Glu Lys Ile
 1570 1575 1580
 Val Ala Gly Arg Val Asn Ile Gln Asp Leu Gln Lys Asn Lys Phe Leu
 1585 1590 1595 1600
 Met Arg Ala Asn Thr Val Asp Asn His Phe
 1605 1610

<210> 3
 <211> 5177
 <212> DNA
 <213> Rattus norvegicus

<400> 3
 ggacagacat ggcacttctg ctgtcttcaa aataataaca ccagaccccc ttcctgttct 60
 ctacattcct gaaagatcta gtctaatacta ggctccaact tttcctccgt cttggagaac 120
 agagatgacc aaagttcagt ttccagctca caactgcctg aaagtccagc tccagaggat 180
 ctgacacact tttctggcct ccatgcaaca catgttgcac aaaaacagac acacacacat 240
 acagagaaga cctccttggg gagacagctt cctggcactg aaaaatcctg accactgtcc 300
 ttgaacatga gtgcccgaag gcaggaatta caggacaggg caatcgtcaa gatagctgct 360
 cacttacccg acctcattgt ctatggagat ttctctcccg agcggccgctc agtgaaatgt 420
 tttgatggag ttctgatgtt tgtcgatatt tcaggcttta ctgcaatgac tgagaagtgc 480
 agcacatcca tgtacatgga cggaggagcc gagcagctgg tggagatcct caactactac 540
 ataagtgcga tagtggagaa agtactgatt ttggaggag acatcctaaa atttgcaggt 600
 gacgccttgt tggccctgtg gaaagtggaa cgaaagcaac tgaagaatat catcacggtg 660
 gtaattaaat gcagcctgga gattcatggc ttgtttgaag ccaaggaggt tgaagaaggc 720
 ctggatatct gagttaagat aggactggct gctggccaca tcaccatgtt ggtctttggg 780
 gatgaaacac ggaactactt cctggtgatt ggccaagcgg tggatgatgt acgccttgct 840
 cagaacatgg ctcatagtaa tgatgttatt ctgtcaccaa actgctggca gctctgtgat 900
 cggagcatga ttgaaatcga gaggattccg gatcagagag cagttaaggt tagcttctta 960
 aaaccacccc caacttttaa cttcgacgag ttttttgcca agtgtatggc cttcattggat 1020
 tattatcctt ctggtgacca caaaaacttc ctaaggcttg cctgcatgct ggagtctgat 1080
 cctgaactcg agttgtctct acaaaagtat gtgatggaaa tcattttgaa gcagattgat 1140
 gacaagcagc ttcggggcta tttatctgag ctctgcctctg tgacgatcgt gtttgtgaac 1200
 ttgatgttta aagagcaaga caaagcagaa gtcataaggat cagccatcca agctgcctgt 1260
 gtgcacatca cttccgtctt gaaggtcttc cgaggccaga tctatggttt tctcatgttt 1320
 gataagggct gctccttcct ctgtgtcttc ggtttccctg gggaaaaggc ccctgacgag 1380
 atcactcacg ctttggaaag tgccgtggat atattcgact tctgctctca ggtccacaaa 1440
 atccgtactg tctccatcgg cgtcgccagt gggattgtct tctgtgggat cgttggacac 1500
 actgtgagac acgagtacac agtcattggc caaaagggtca atattgctgc caggatgatg 1560
 atgtattacc caggcatcgt gacctgcgac tctgtcacat acgatggcag caacctgcca 1620
 gcctactttt ttaaagagct tccaaagaaa gtcatagaa gagttgcga tcccggacca 1680
 gtgtatcagt gtctggcct caatgagaaa gtcattgttg gtatggccta tctcatctgc 1740
 aacagatatg agggctaccc tttgctgggt cgtgttaggg agatcgacta tttcatgtct 1800
 actatgaagg actttctgat gacgaactgc agccgagttc taatgtatga aggattgcca 1860
 ggatatggga aaagccaggt acttatggaa atcgagtatc tggcctccca gcatgagaac 1920
 catagggtctg ttgctattgc actgactaag atcagcttcc atcaaaattt ttacactatc 1980
 cagatactca tggctaactg actaggtctg gatacttgta aacattacaa agaacgacag 2040
 accaatcttc aaaatagagt caagacgctg ttggatgata aataccactg tctccttaac 2100
 gacatcttcc atgttcagtt ccccgtttcc cgggagatgt ccaggatgag caagataaga 2160
 aagcagaagc aactggaagc tctgtttatg aagatcctgg agcaaacagt gagggaagaa 2220
 aggattatct tcatcatcga cgaggcccag tttgtggacg tagcctcctg ggccttcata 2280
 gaaaagctca tccggtccat gcccatcttc attgttatgt cctgtgttcc cttccctgaa 2340
 actcctctcg cagctgccaa tgccataatg aagaaccgga acaccaccta catcacactc 2400
 ggtacctagc agcctcagga aatccgggac aaggtctgtg ttgacctgag tgtaagcagc 2460
 atccccagag agccttgactc gtacctgtgt gagggagct gcgggattcc gtattactgt 2520
 gaggaactgc tgaaaaacct cgaccaccac agaattctca ttttccaaca agcagaggct 2580
 gagggaaaaga caaacgtgac ctggaataac ctgttcaagt actctgttaa gccgacagaa 2640
 gacatgtatc tgtatacttc catagccgag ggacagaaaag aagcctgtta ccttacaagt 2700
 ggtgtcagac taaagaactt gtcacctcca gcactcgtca aagaaatctc tctggttcaa 2760
 ctggacagca tgagcctttc ccatcagatg ctggtgaggt gtgctgctat cattggtcta 2820
 accttcacca cagagctgct gtttgagatt ctcccctgct ggaacatgaa gatgatgatc 2880
 aaggccctgg ccaccctagt ggaatcaaat gtctttgatt gctttcggag tagcaaagac 2940
 cttcaactag ccttaaagca aaacgtgacc acgtttgaag ttcattatcg ctctttgtcc 3000

ctgaagtcca	aggaaggggtt	agcttacagt	gaggaggagc	agctccgtga	aatggaagga	3060
gaggtgattg	aatgccgcat	ccttcgggttc	tgcagaccca	taatgcagaa	gacagcctac	3120
gaactgtggc	tcaaggacca	gaagaaagtc	ttgcatctga	aatgcgcccc	ctttttggag	3180
gagagtgcc	atcgggtgcaa	ccactgcaga	aacagagact	tcattcctta	ccaccacttc	3240
atagcggaca	ttcgactcaa	cactctggac	atggatactg	tcaagaagat	ggtgaagtcc	3300
cacggattta	aaactgaaga	cgaggtcatc	ttttctaaat	cagagatccc	caggaaattc	3360
aaattccccg	agaacatcag	catcacagaa	acaagggaaa	aaatcttgca	tttctttgac	3420
aatgttatca	taaagatgag	gacgtctcag	gatgatgtca	tccctctaga	atcgtgccat	3480
tgtgaggagc	tgctccagat	tgctcatctg	cctctggccc	agcatttcgt	agccttagaa	3540
gaaaacaaca	aagccttgta	ctacttccta	gaacttgcat	ctgcctatct	catcctggga	3600
gacaactata	acgcatacat	gtattttggc	gaaggggaaa	ggctgttgaa	atctctgaca	3660
aatgaagatt	cttgaggatca	gacctttgaa	tatgctacgt	tttatagtct	caaaggtgag	3720
atctgtttta	atatgggaca	gatggtgctc	gccaagaaaa	tgctgagaaa	agcactgaag	3780
cttctcaaca	gaatgtttcc	ctgcaatcta	ctctccctga	ctttccaaat	gcacattgag	3840
aaaaacagac	tctcccactt	catgaaccag	catacccagg	agggctcgct	gccaggggaag	3900
aagctggccc	aacttttcct	gcagtcgtcc	tgcttctccc	tgctgtggaa	gatctatagc	3960
ttgaacttct	ttttccacta	caagtactat	ggctcgtctg	cagcaataat	gcagatgaac	4020
acctcgttag	aaactcaaaa	caattttccag	atcatcaagg	ctttcctgga	cttttccttg	4080
taccgccatc	tggtctggata	cgagggcgtg	tggttcaaat	atgaaatcct	ggtcatggag	4140
cagctcttga	acctccccct	gaaaggcgaa	gcctttgaaa	tcattggccta	tgccagccgac	4200
gcactgggcc	atatcaagtt	cttaaccggt	catctggact	tgccatttga	attaggctcc	4260
cgagctcaca	agatgtggtc	acttctccgg	aatcccaaca	aataccatat	ggttctctgc	4320
agactgagta	aacctctttt	cttgaagagc	agatacaagc	atttggtcca	ggtgctggga	4380
tggttggtgg	acctttctgt	aacagaggag	cacatcttca	gcaaggcatt	tttctatttc	4440
gtctgcttgg	acatcatgct	ttattctggc	ttcattttaca	gaacatttga	agaatgtttg	4500
gaattcatac	accacaatga	agacaacaga	atcctcaagt	tccaaagcgg	actcctcctg	4560
ggactttact	cctgcatagc	tgtctggtac	gccagacttc	aggaatggga	caacttttac	4620
aaattttcca	atagagcgaa	gacttttagt	actcgaagaa	ccccaacggt	cctttactac	4680
gaaggaatth	ctaggtatat	ggaagggcaa	gtcctccatc	ttcagaagca	aatagaagag	4740
caggccgaga	atgctcagga	cagtgggggtg	gagctactta	aggccttaga	gacccttggt	4800
gctcaaaaata	ccactggccc	cgtcttctac	cccaggctct	accatttgat	ggcctatgtc	4860
tgtatactga	tgggagacgg	gcacagttgt	gacttcttcc	taaacacagc	cttggagctc	4920
tctgagacac	aggggaattt	gctggagaaa	tgttggtcga	gcattgagtaa	ggaatgggtg	4980
tactcagccc	ccgagttgac	aggagatcaa	tggttccaga	cagtcttgag	tctcccatcg	5040
tgggataaaa	ttgtatcagg	caacgtaacc	cttcaggatg	ttcaaaagaa	caaattcttg	5100
atgagagtta	atattctgga	caatcctttc	taataattat	gaatgagaac	aaagattgca	5160
aaaaaaaaaa	aaaaaaaa					5177

<210> 4

<211> 1608

<212> PRT

<213> Rattus norvegicus

<400> 4

Met	Ser	Ala	Arg	Arg	Gln	Glu	Leu	Gln	Asp	Arg	Ala	Ile	Val	Lys	Ile
1				5				10						15	
Ala	Ala	His	Leu	Pro	Asp	Leu	Ile	Val	Tyr	Gly	Asp	Phe	Ser	Pro	Glu
			20					25					30		
Arg	Pro	Ser	Val	Lys	Cys	Phe	Asp	Gly	Val	Leu	Met	Phe	Val	Asp	Ile
			35				40					45			
Ser	Gly	Phe	Thr	Ala	Met	Thr	Glu	Lys	Phe	Ser	Thr	Ala	Met	Tyr	Met
			50			55					60				
Asp	Arg	Gly	Ala	Glu	Gln	Leu	Val	Glu	Ile	Leu	Asn	Tyr	Tyr	Ile	Ser
65				70				75						80	
Ala	Ile	Val	Glu	Lys	Val	Leu	Ile	Phe	Gly	Gly	Asp	Ile	Leu	Lys	Phe
				85				90						95	
Ala	Gly	Asp	Ala	Leu	Leu	Ala	Leu	Trp	Lys	Val	Glu	Arg	Lys	Gln	Leu
			100					105					110		
Lys	Asn	Ile	Ile	Thr	Val	Val	Ile	Lys	Cys	Ser	Leu	Glu	Ile	His	Gly
			115				120						125		

Leu	Phe	Glu	Ala	Lys	Glu	Val	Glu	Glu	Gly	Leu	Asp	Ile	Arg	Val	Lys
130						135					140				
Ile	Gly	Leu	Ala	Ala	Gly	His	Ile	Thr	Met	Leu	Val	Phe	Gly	Asp	Glu
145					150					155					160
Thr	Arg	Asn	Tyr	Phe	Leu	Val	Ile	Gly	Gln	Ala	Val	Asp	Asp	Val	Arg
				165					170						175
Leu	Ala	Gln	Asn	Met	Ala	Gln	Met	Asn	Asp	Val	Ile	Leu	Ser	Pro	Asn
			180					185					190		
Cys	Trp	Gln	Leu	Cys	Asp	Arg	Ser	Met	Ile	Glu	Ile	Glu	Arg	Ile	Pro
		195					200					205			
Asp	Gln	Arg	Ala	Val	Lys	Val	Ser	Phe	Leu	Lys	Pro	Pro	Pro	Thr	Phe
		210				215					220				
Asn	Phe	Asp	Glu	Phe	Phe	Ala	Lys	Cys	Met	Ala	Phe	Met	Asp	Tyr	Tyr
225					230					235					240
Pro	Ser	Gly	Asp	His	Lys	Asn	Phe	Leu	Arg	Leu	Ala	Cys	Met	Leu	Glu
				245					250					255	
Ser	Asp	Pro	Glu	Leu	Glu	Leu	Ser	Leu	Gln	Lys	Tyr	Val	Met	Glu	Ile
			260					265					270		
Ile	Leu	Lys	Gln	Ile	Asp	Asp	Lys	Gln	Leu	Arg	Gly	Tyr	Leu	Ser	Glu
		275					280					285			
Leu	Arg	Pro	Val	Thr	Ile	Val	Phe	Val	Asn	Leu	Met	Phe	Lys	Glu	Gln
		290				295					300				
Asp	Lys	Ala	Glu	Val	Ile	Gly	Ser	Ala	Ile	Gln	Ala	Ala	Cys	Val	His
305					310					315					320
Ile	Thr	Ser	Val	Leu	Lys	Val	Phe	Arg	Gly	Gln	Ile	Asn	Lys	Val	Phe
				325					330					335	
Met	Phe	Asp	Lys	Gly	Cys	Ser	Phe	Leu	Cys	Val	Phe	Gly	Phe	Pro	Gly
			340					345					350		
Glu	Lys	Ala	Pro	Asp	Glu	Ile	Thr	His	Ala	Leu	Glu	Ser	Ala	Val	Asp
		355					360					365			
Ile	Phe	Asp	Phe	Cys	Ser	Gln	Val	His	Lys	Ile	Arg	Thr	Val	Ser	Ile
		370				375					380				
Gly	Val	Ala	Ser	Gly	Ile	Val	Phe	Cys	Gly	Ile	Val	Gly	His	Thr	Val
385					390					395					400
Arg	His	Glu	Tyr	Thr	Val	Ile	Gly	Gln	Lys	Val	Asn	Ile	Ala	Ala	Arg
				405					410					415	
Met	Met	Met	Tyr	Tyr	Pro	Gly	Ile	Val	Thr	Cys	Asp	Ser	Val	Thr	Tyr
			420					425					430		
Asp	Gly	Ser	Asn	Leu	Pro	Ala	Tyr	Phe	Phe	Lys	Glu	Leu	Pro	Lys	Lys
		435					440					445			
Val	Met	Lys	Gly	Val	Ala	Asp	Pro	Gly	Pro	Val	Tyr	Gln	Cys	Leu	Gly
		450				455					460				
Leu	Asn	Glu	Lys	Val	Met	Phe	Gly	Met	Ala	Tyr	Leu	Ile	Cys	Asn	Arg
465					470					475					480
Tyr	Glu	Gly	Tyr	Pro	Leu	Leu	Gly	Arg	Val	Arg	Glu	Ile	Asp	Tyr	Phe
				485					490					495	
Met	Ser	Thr	Met	Lys	Asp	Phe	Leu	Met	Thr	Asn	Cys	Ser	Arg	Val	Leu
			500					505					510		
Met	Tyr	Glu	Gly	Leu	Pro	Gly	Tyr	Gly	Lys	Ser	Gln	Val	Leu	Met	Glu
		515					520					525			
Ile	Glu	Tyr	Leu	Ala	Ser	Gln	His	Glu	Asn	His	Arg	Ala	Val	Ala	Ile
		530				535					540				
Ala	Leu	Thr	Lys	Ile	Ser	Phe	His	Gln	Asn	Phe	Tyr	Thr	Ile	Gln	Ile
545					550					555					560
Leu	Met	Ala	Asn	Val	Leu	Gly	Leu	Asp	Thr	Cys	Lys	His	Tyr	Lys	Glu
				565					570					575	
Arg	Gln	Thr	Asn	Leu	Gln	Asn	Arg	Val	Lys	Thr	Leu	Leu	Asp	Asp	Lys
			580					585					590		
Tyr	His	Cys	Leu	Leu	Asn	Asp	Ile	Phe	His	Val	Gln	Phe	Pro	Val	Ser
		595					600					605			
Arg	Glu	Met	Ser	Arg	Met	Ser	Lys	Ile	Arg	Lys	Gln	Lys	Gln	Leu	Glu
	610					615					620				

Ala	Leu	Phe	Met	Lys	Ile	Leu	Glu	Gln	Thr	Val	Arg	Glu	Glu	Arg	Ile
625					630					635					640
Ile	Phe	Ile	Ile	Asp	Glu	Ala	Gln	Phe	Val	Asp	Val	Ala	Ser	Trp	Ala
				645					650						655
Phe	Ile	Glu	Lys	Leu	Ile	Arg	Ser	Met	Pro	Ile	Phe	Ile	Val	Met	Ser
			660					665					670		
Leu	Cys	Pro	Phe	Pro	Glu	Thr	Pro	Cys	Ala	Ala	Ala	Asn	Ala	Ile	Met
		675					680					685			
Lys	Asn	Arg	Asn	Thr	Thr	Tyr	Ile	Thr	Leu	Gly	Thr	Met	Gln	Pro	Gln
	690					695					700				
Glu	Ile	Arg	Asp	Lys	Val	Cys	Val	Asp	Leu	Ser	Val	Ser	Ser	Ile	Pro
705					710					715					720
Arg	Glu	Leu	Asp	Ser	Tyr	Leu	Val	Glu	Gly	Ser	Cys	Gly	Ile	Pro	Tyr
			725					730						735	
Tyr	Cys	Glu	Glu	Leu	Leu	Lys	Asn	Leu	Asp	His	His	Arg	Ile	Leu	Ile
			740					745					750		
Phe	Gln	Gln	Ala	Glu	Ala	Glu	Glu	Lys	Thr	Asn	Val	Thr	Trp	Asn	Asn
		755					760					765			
Leu	Phe	Lys	Tyr	Ser	Val	Lys	Pro	Thr	Glu	Asp	Met	Tyr	Leu	Tyr	Thr
	770					775					780				
Ser	Ile	Ala	Ala	Gly	Gln	Lys	Glu	Ala	Cys	Tyr	Leu	Thr	Ser	Gly	Val
785					790					795					800
Arg	Leu	Lys	Asn	Leu	Ser	Pro	Pro	Ala	Ser	Leu	Lys	Glu	Ile	Ser	Leu
			805					810						815	
Val	Gln	Leu	Asp	Ser	Met	Ser	Leu	Ser	His	Gln	Met	Leu	Val	Arg	Cys
			820					825					830		
Ala	Ala	Ile	Ile	Gly	Leu	Thr	Phe	Thr	Thr	Glu	Leu	Leu	Phe	Glu	Ile
		835					840					845			
Leu	Pro	Cys	Trp	Asn	Met	Lys	Met	Met	Ile	Lys	Ala	Leu	Ala	Thr	Leu
	850					855					860				
Val	Glu	Ser	Asn	Val	Phe	Asp	Cys	Phe	Arg	Ser	Ser	Lys	Asp	Leu	Gln
865					870					875					880
Leu	Ala	Leu	Lys	Gln	Asn	Val	Thr	Thr	Phe	Glu	Val	His	Tyr	Arg	Ser
			885						890					895	
Leu	Ser	Leu	Lys	Ser	Lys	Glu	Gly	Leu	Ala	Tyr	Ser	Glu	Glu	Glu	Gln
			900					905					910		
Leu	Arg	Glu	Met	Glu	Gly	Glu	Val	Ile	Glu	Cys	Arg	Ile	Leu	Arg	Phe
		915					920					925			
Cys	Arg	Pro	Ile	Met	Gln	Lys	Thr	Ala	Tyr	Glu	Leu	Trp	Leu	Lys	Asp
		930				935					940				
Gln	Lys	Lys	Val	Leu	His	Leu	Lys	Cys	Ala	Arg	Phe	Leu	Glu	Glu	Ser
945					950					955					960
Ala	His	Arg	Cys	Asn	His	Cys	Arg	Asn	Arg	Asp	Phe	Ile	Pro	Tyr	His
			965						970					975	
His	Phe	Ile	Ala	Asp	Ile	Arg	Leu	Asn	Thr	Leu	Asp	Met	Asp	Thr	Val
			980					985					990		
Lys	Lys	Met	Val	Lys	Ser	His	Gly	Phe	Lys	Thr	Glu	Asp	Glu	Val	Ile
		995					1000					1005			
Phe	Ser	Lys	Ser	Glu	Ile	Pro	Arg	Lys	Phe	Lys	Phe	Pro	Glu	Asn	Ile
	1010					1015					1020				
Ser	Ile	Thr	Glu	Thr	Arg	Glu	Lys	Ile	Leu	His	Phe	Phe	Asp	Asn	Val
1025					1030					1035					1040
Ile	Ile	Lys	Met	Arg	Thr	Ser	Gln	Asp	Asp	Val	Ile	Pro	Leu	Glu	Ser
			1045						1050					1055	
Cys	His	Cys	Glu	Glu	Leu	Leu	Gln	Ile	Val	Ile	Leu	Pro	Leu	Ala	Gln
			1060					1065					1070		
His	Phe	Val	Ala	Leu	Glu	Glu	Asn	Lys	Ala	Leu	Tyr	Tyr	Phe	Leu	
		1075					1080				1085				
Glu	Leu	Ala	Ser	Ala	Tyr	Leu	Ile	Leu	Gly	Asp	Asn	Tyr	Asn	Ala	Tyr
	1090					1095					1100				
Met	Tyr	Leu	Gly	Glu	Gly	Glu	Arg	Leu	Leu	Lys	Ser	Leu	Thr	Asn	Glu
1105					1110					1115					1120

Asp Ser Trp Ser Gln Thr Phe Glu Tyr Ala Thr Phe Tyr Ser Leu Lys
 1125 1130 1135
 Gly Glu Ile Cys Phe Asn Met Gly Gln Met Val Leu Ala Lys Lys Met
 1140 1145 1150
 Leu Arg Lys Ala Leu Lys Leu Leu Asn Arg Met Phe Pro Cys Asn Leu
 1155 1160 1165
 Leu Ser Leu Thr Phe Gln Met His Ile Glu Lys Asn Arg Leu Ser His
 1170 1175 1180
 Phe Met Asn Gln His Thr Gln Glu Gly Ser Leu Pro Gly Lys Lys Leu
 1185 1190 1195 1200
 Ala Gln Leu Phe Leu Gln Ser Ser Cys Phe Ser Leu Leu Trp Lys Ile
 1205 1210 1215
 Tyr Ser Leu Asn Phe Phe Phe His Tyr Lys Tyr Tyr Gly Arg Leu Ala
 1220 1225 1230
 Ala Ile Met Gln Met Asn Thr Ser Leu Glu Thr Gln Asn Asn Phe Gln
 1235 1240 1245
 Ile Ile Lys Ala Phe Leu Asp Phe Ser Leu Tyr Arg His Leu Ala Gly
 1250 1255 1260
 Tyr Glu Gly Val Trp Phe Lys Tyr Glu Ile Leu Val Met Glu Gln Leu
 1265 1270 1275 1280
 Leu Asn Leu Pro Leu Lys Gly Glu Ala Phe Glu Ile Met Ala Tyr Ala
 1285 1290 1295
 Ala Asp Ala Leu Gly His Ile Lys Phe Leu Thr Gly His Leu Asp Leu
 1300 1305 1310
 Ala Ile Glu Leu Gly Ser Arg Ala His Lys Met Trp Ser Leu Leu Arg
 1315 1320 1325
 Asn Pro Asn Lys Tyr His Met Val Leu Cys Arg Leu Ser Lys Pro Leu
 1330 1335 1340
 Phe Leu Lys Ser Arg Tyr Lys His Leu Val Gln Val Leu Gly Trp Leu
 1345 1350 1355 1360
 Trp Asp Leu Ser Val Thr Glu Glu His Ile Phe Ser Lys Ala Phe Phe
 1365 1370 1375
 Tyr Phe Val Cys Leu Asp Ile Met Leu Tyr Ser Gly Phe Ile Tyr Arg
 1380 1385 1390
 Thr Phe Glu Glu Cys Leu Glu Phe Ile His His Asn Glu Asp Asn Arg
 1395 1400 1405
 Ile Leu Lys Phe Gln Ser Gly Leu Leu Leu Gly Leu Tyr Ser Cys Ile
 1410 1415 1420
 Ala Val Trp Tyr Ala Arg Leu Gln Glu Trp Asp Asn Phe Tyr Lys Phe
 1425 1430 1435 1440
 Ser Asn Arg Ala Lys Thr Leu Val Thr Arg Arg Thr Pro Thr Val Leu
 1445 1450 1455
 Tyr Tyr Glu Gly Ile Ser Arg Tyr Met Glu Gly Gln Val Leu His Leu
 1460 1465 1470
 Gln Lys Gln Ile Glu Glu Gln Ala Glu Asn Ala Gln Asp Ser Gly Val
 1475 1480 1485
 Glu Leu Leu Lys Ala Leu Glu Thr Leu Val Ala Gln Asn Thr Thr Gly
 1490 1495 1500
 Pro Val Phe Tyr Pro Arg Leu Tyr His Leu Met Ala Tyr Val Cys Ile
 1505 1510 1515 1520
 Leu Met Gly Asp Gly His Ser Cys Asp Phe Phe Leu Asn Thr Ala Leu
 1525 1530 1535
 Glu Leu Ser Glu Thr Gln Gly Asn Leu Leu Glu Lys Cys Trp Leu Ser
 1540 1545 1550
 Met Ser Lys Glu Trp Trp Tyr Ser Ala Pro Glu Leu Thr Gly Asp Gln
 1555 1560 1565
 Trp Leu Gln Thr Val Leu Ser Leu Pro Ser Trp Asp Lys Ile Val Ser
 1570 1575 1580
 Gly Asn Val Thr Leu Gln Asp Val Gln Lys Asn Lys Phe Leu Met Arg
 1585 1590 1595 1600
 Val Asn Ile Leu Asp Asn Pro Phe
 1605

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/26129

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12Q 1/00; C12N 9/88, 1/20, 15/00; C07H 21/04; C07K 1/00

US CL :424/94.5; 435/4, 232, 252.3, 320.1; 536/23.2; 530/350

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/94.5; 435/4, 232, 252.3, 320.1; 536/23.2; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

West and STN - stn files included caplus, medline, biosis, embase, biotechds and scisearch

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	US 6,001,553 A (BROACH ET AL.) 14 December 1999, see the entire document.	1-16
A,P	WICKER, R. et al. Cloning and expression of human adenylyl cyclase type VI in normal thyroid tissue. Biochimica et Biophysica Acta. 07 September 2000, Vol. 1493, pages 279-283, see the entire document.	1-16
Y	HELLEVUO, K. et al. The Characterization of a Novel Human Adenylyl Cyclase Which is Present in Brain and Other Tissues. The Journal of Biological Chemistry. 12 May 1995, Vol. 270, No. 19, pages 11581-11589, see the entire document.	1-16

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

14 NOVEMBER 2000

Date of mailing of the international search report

29 DEC 2000

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

Dorothea Lawrence
TERCHAND SAIDHA

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/26129

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-16

Remark on Protest

☐
☐

- The additional search fees were accompanied by the applicant's protest.
No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/26129

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-16, drawn to a polypeptide (soluble human adenylyl cyclase activity), composition comprising the polypeptide and polynucleotide encoding the polypeptide, vector and host cell.

Group II, claim(s) 17-19, drawn to antibody.

Group III, claim(s) 20-22, drawn to a method of identifying a substance which modulates soluble human adenylyl cyclase.

Group IV, claims 23-29, drawn to a method of inhibiting spermatogenesis in a male, administering a substance that inhibit soluble adenylyl cyclase activity.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Group I has a special technical feature of nucleotide sequence encoding human adenylyl cyclase which Groups II-IV do not share; Group II has a special technical feature of an antibody which Groups I & III-IV do not share; Group III has a special technical feature of a method of identification of a substance that modulate human adenylyl cyclase which Groups I-II & IV do not share; Group IV has a special technical feature of a method of inhibiting spermatogenesis in a male by inhibiting human adenylyl cyclase which Groups I-III do not share.